10/675,567
2-9-4

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 21/07, B01L 3/00, G01N 33/487 — A1

(11) International Publication Number: **WO 97/21090**

(43) International Publication Date: 12 June 1997 (12.06.97)

(21) International Application Number: PCT/US96/19514

(22) International Filing Date: 5 December 1996 (05.12.96)

(30) Priority Data:

60/008,215	5 December 1995 (05.12.95)	US
60/008,267	6 December 1995 (06.12.95)	US
60/008,819	18 December 1995 (18.12.95)	US
60/023,756	12 August 1996 (12.08.96)	US

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(71) Applicant: GAMERA BIOSCIENCE [US/US]; 26 Lansdowne Street, Cambridge, MA 02139 (US).

(72) Inventors: MIAN, Alec; 137 Magazine Street, Cambridge, MA 03139 (US). KIEFFER-HIGGINS, Stephen, G.; 30 Beaumont Street, Dorchester, MA 02124 (US). COREY, George, D.; 65 Harding Street, Newton, MA 02165 (US).

(74) Agent: NOONAN, Kevin, E.; McDonnell Boehnen Hulbert & Berghoff, Suite 700, 300 South Wacker Drive, Chicago, IL 60606 (US).

(54) Title: DEVICES AND METHODS FOR USING CENTRIPETAL ACCELERATION TO DRIVE FLUID MOVEMENT IN A MICROFLUIDICS SYSTEM WITH ON-BOARD INFORMATICS

(57) Abstract

This invention relates to methods and apparatus for performing microanalytic and microsynthetic analyses and procedures. The invention provides a microsystem platform and a micromanipulation device for manipulating the platform that utilizes the centripetal force resulting from rotation of the platform to motivate fluid movement through microchannels. The microsystem platforms of the invention are also provided having system informatics and data acquisition, analysis and storage and retrieval informatics encoded on the surface of the disk opposite to the surface containing the fluidic components. Methods specific for the apparatus of the invention for performing any of a wide variety of microanalytical or microsynthetic processes are provided.

Devices and Methods for Using Centripetal Acceleration to Drive Fluid Movement in a Microfluidics System with On-board Informatics

This application claims priority to U.S. Provisional Applications, Serial Nos. 60/008,215, filed December 5, 1995, 60/008,267, filed December 6, 1995, 60/008,819, filed December 18, 1995, and 60/023,756, filed August 12, 1996, the disclosures of each of which are explicitly incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to methods and apparatus for performing microanalytic and microsynthetic analyses and procedures. In particular, the invention relates to microminiaturization of genetic, biochemical and chemical processes related to analysis, synthesis and purification. Specifically, the invention provides a microsystem platform and a micromanipulation device to manipulate the platform by rotation, thereby utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement through microchannels embedded in the microplatform. The microsystem platforms of the invention are also provided having system informatics and data acquisition, analysis and storage and retrieval informatics encoded on the surface of the disk opposite to the surface containing the fluidic components. Methods for performing any of a wide variety of microanalytical or microsynthetic processes using the microsystems apparatus of the invention are also provided.

2. Background of the Related Art

In the field of medical, biological and chemical assays, a mechanical and automated fluid handling systems and instruments produced to operate on a macroscopic (*i.e.*, milliliters and milligrams) scale are known in the prior art.

U.S. Patent 4, 279,862, issued July 21, 1981 to Bertaudiere *et al.* Disclose a centrifugal photometric analyzer.

U.S. Patent 4,381,291, issued April 26, 1983 to Ekins teach analytic measurement of free ligands.

U.S. Patent 4,515,889, issued May 7, 1985 to Klose *et al.* teach automated mixing and incubating reagents to perform analytical determinations.

U.S. Patent 4,676,952, issued June 30, 1987 to Edelmann *et al.* teach a

Lee *et al.*, 1978, *Clin. Chem.* 24: 1361-1365 teach an automated blood fractionation system.

Cho *et al.*, 1982, *Clin. Chem.* 28: 1965-1961 teach a multichannel electrochemical centrifugal analyzer.

Bertrand *et al.*, 1982, *Clinica Chimica Acta* 119: 275-284 teach automated determination of serum 5' -nucleotidase using a centrifugal analyzer.

Schembri *et al.*, 1992, *Clin. Chem.* 38: 1665-1670 teach a portable whole blood analyzer.

Walters *et al.*, 1995, Basic Medical Laboratory Technologies, 3rd ed., Delmar Publishers: Boston teach a variety of automated medical laboratory analytic techniques.

Recently, microanalytical devices for performing select reaction pathways have been developed.

U.S. Patent 5,006,749, issued April 9, 1991 to White disclose methods and apparatus for using ultrasonic energy to move microminiature elements.

U.S. Patent No. 5,252,294, issued October 12, 1993 to Kroy *et al.* teach a micromechanical structure for performing certain chemical microanalyses.

U.S. Patent 5,304,487, issued April 19, 1994 to Wilding *et al.* teach fluid handling on microscale analytical devices.

U.S. Patent 5,368,704 issued November 29, 1994 to Madou *et al.* teach microelectrochemical valves.

International Application, Publication No. WO93/22053, published 11 November 1993 to University of Pennsylvania disclose microfabricated detection structures.

International Application, Publication No. WO93/22058, published 11 November 1993 to University of Pennsylvania disclose microfabricated structures for performing polynucleotide amplification.

Columbus *et al.*, 1987, *Clin. Chem.* 33: 1531-1537 teach fluid management of biological fluids.

Ekins *et al.*, 1992, *Ann. Biol. Clin.* 50: 337-353 teach a multianalytical microspot immunoassay.

Wilding *et al.*, 1994, *Clin. Chem.* 40: 43-47 disclose manipulation of fluids on straight channels micromachined into silicon.

In one aspect of the invention is provided a microanalytic/microsynthetic system comprising a combination of two elements. The first element is a microplatform that is a rotatable structure, most preferably a disk, the disk comprising sample, inlet ports, fluid microchannels, reagent reservoirs, reaction chambers, detection chambers and sample outlet ports. The disk is rotated at speeds from about 1-30,000 rpm for generating centripetal acceleration that enables fluid movement. The disks of the invention also preferably comprise fluid inlet ports, air outlet ports and air displacement channels. The fluid inlet ports allow samples to enter the disk for processing and/or analysis. The air outlet ports and in particular the air displacement ports provide a means for fluids to displace air, thus ensuring uninhibited movement of fluids on the disk. Specific sites on the disk also preferably comprise elements that allow fluids to be analyzed, including thermal sources, light, particularly monochromatic light, sources, and acoustic sources, as well as detectors for each of these effectors. Alternatively, some or all of these elements can be contained on a second disk that is placed in optical or direct physical contact with the first.

The second element of the invention is a micromanipulation device that is a disk player/ reader device that controls the function of the disk. This device comprises mechanisms and motors that enable the disk to be loaded and spun. In addition, the device provides means for a user to operate the microsystems in the disk and access and analyze data, preferably using a keypad and computer display.

The invention provides methods and apparatus for the manipulation of samples consisting of fluids, cells and/or particles containing or comprising an analyte. The microplatform disks of the invention comprise microsystems composed of, but no restricted to, sample input ports, microchannels, chambers, valves, heaters, chillers, electrophoretic and detection systems upon a disk. Movement of the sample is facilitated by the judicious incorporation of air holes and air displacement channels that allow air to be displaced but prevent fluid and/or particle loss upon acceleration.

A preferred embodiment of the disk of the invention incorporates micromachined mechanical, optical, and fluidic control structures (or "systems") on a substrate that is preferably made from plastic, silica, quartz, metal or ceramic. These structures are constructed on a sub-millimeter scale by photolithography, etching, stamping or other appropriate means.

Sample movement is controlled by centripetal or linear acceleration and the

Figure 6 is a schematic diagram of a piezoelectric stack microvalve.

Figure 7 is a schematic diagram of a pneumatically-activated microvalve.

Figure 8 is a schematic diagram of device to deliver pneumatic pressure to a revolving disk.

Figure 9 is a schematic diagram of a bimetallic microvalve.

Figure 10 is a schematic diagram of a pressure-balanced microvalve.

Figure 11 is a schematic diagram of a polymeric relaxation microvalve.

Figures 12A and 12B represent two different embodiments of fluorescence detectors of the invention.

Figures 13A, 13B and 13C are a schematic diagrams of a multiple loading device for the disk.

Figures 14A through 14F illustrate laser light-activated CD-ROM capability of the disk of the invention.

Figure 15 is a flow diagram of the processor control structure of a player/reader device of the invention.

Figure 16 is a schematic diagram of a transverse spectroscopic detection chamber. Figures 17A through 17E are schematic diagrams of the different structural and functional layers of a disk of the invention configured for DNA sequencing.

Figure 17F is a schematic diagram of basic zones and design formats for analytic disks.

Figure 17G is a schematic diagram of a disk configured as a home test diagnostic disk.

Figure 17H is a schematic diagram of a disk configured as a simplified immunocapacitance assay.

Figure 17I is a schematic diagram of a disk configured as a gas and particle disk.

Figure 17J is a schematic diagram of a hybrid disk comprising separately-assembled chips.

Figure 17K is a schematic diagram of a sample authorizing disk.

Figure 17L is a schematic diagram of a disk configured for pathological applications.

Figure 17M is a schematic diagram of a disk with removable assay layers.

Figure 17N is a schematic diagram of a disk for assaying aerosols.

performing microanalytical and microsynthetic assays of biological, chemical, environmental and industrial samples. For the purposes of this invention, the term "sample" will be understood to encompass any chemical or particulate species of interest, either isolated or detected as a constituent of a more complex mixture, or synthesized from precursor species. The invention provides a combination of a microplatform that is a rotatable, analytic/synthetic microvolume assay platform (collectively referred to herein as a "disk") and a micromanipulation device for manipulating the platform to achieve fluid movement on the platform arising from centripetal force on the platform as result of rotation. The platform of the invention is preferably and advantageously a circular disk; however, any platform capable of being rotated to impart centripetal for a fluid on the platform is intended to fall within the scope of the invention.

The microplatforms of the invention (preferably and hereinafter collectively referred to as "disks"; for the purposes of this invention, the terms "microplatform", "microsystems platform" and "disk" are considered to be interchangeable), are provided to comprise one or a multiplicity of microsynthetic or microanalytic systems. Such microsynthetic or microanalytic systems in turn comprise combinations of related components as described in further detail herein that are operably interconnected to allow fluid flow between components upon rotation of the disk. These components can be fabricated as described below either integral to the disk or as modules attached to, placed upon, in contact with or embedded in the disk. The invention also comprises a micromanipulation device for manipulating the disks of the invention, wherein the disk is rotated within the device to provide centripetal force to effect fluid flow on the disk. Accordingly, the device provides means for rotating the disk at a controlled rotational velocity, for stopping and starting disk rotation, and advantageously for changing the direction of rotation of the disk. Both electromechanical means and control means, as further described herein, are provided as components of the devices of the invention. User interface means (such as a keypad and a display) are also provided.

The invention provides methods and apparatus for the manipulation of samples consisting of fluids, cells and/or particles (generically termed "sample" herein) containing an analyte of interest. The platforms of the invention consist of systems comprising sample input ports, microchannels for fluid flow, reagent reservoirs, mixing chambers, reaction chambers, optical reading chambers, valves for controlling fluid flow

outlet port by the controlled opening of microvalves in the reaction chamber.

Analytical arrays constituting components of the microplatforms of the invention include detection systems for detecting, monitoring, quantitating or analyzing reaction course, products or side-products. Detection systems useful in the fabrication and use of the microplatforms of the invention include, but are not limited to, fluorescent, chemiluminescent, colorimetric, electrochemical and radioactivity detecting means. Optionally, the detection system can be integral to the platform, comprise a component of the device manipulating the platform, or both.

Thus, the microplatform and micromanipulation device provided by the invention produce analytic or synthetic data to be processed. Data processing is accomplished either by a processor and memory module on the disk, by the device microprocessor and memory, or by an out board computer connected to the micromanipulation device. Removable media for data retrieval and storage is provided either by the disk itself or by the device, using computer diskette, tape, or optical media. Alternatively and advantageously, data is written on the microplatform using CD-read/write technologies and conventional optical data storage systems In such embodiments, data is written to the microplatform on the underside of the platform, opposite to the "wet" chemistry side holding the various microsystem components disclosed herein..

The physical parameters of the microplatforms of the invention are widely variable. When provided as a disk, the disk radius ranges from 1-25cm, and disk thickness ranges from 0.1mm to 10cm, more preferably 0.1 to 100mm. Preferred embodiments that are most advantageous for manufacturing and operation of the disks of the invention have dimensions within one or more of four pre-existing formats: (1) 3-inch compact disk (CD), having a radius of about 3.8cm and thickness of about 1mm; (2) 5-inch CD, having a radius of about 6cm and a thickness of 1mm; (3) 8-inch CDV (commercially termed a "Laservision" disk), having a radius of 10cm and a thickness of 2mm; and (4) 12-inch CDV disk, having a radius of 15cm and a thickness of 2mm.

Microchannel and reservoir sizes are optimally determined by specific applications and by the amount of reagent and reagent delivery rates required for each particular embodiment of the microanalytic and micro synthetic methods of the invention. For microanalytical applications, for example, disk dimensions of a 5-in CD (6cm x 1mm) are preferred, allowing reagent reservoirs to contain up to 0.5ml. (close

force is a function of platform radius, disk rotation speed and fluid density. Certain functional parameters relevant to the platform microsystems of this invention are understood in terms of the following equations. These should represent limits of system performance, because they assume both viscous and non-viscous (turbulent) losses for fully-developed fluid flow.

The driving force for fluid motion or creating fluid pressures is the force on matter which results from centripetal acceleration. A device may rotate at an angular rate of f in revolutions/sec and angular frequency

$$\omega = 2\pi f \quad (1)$$

The centripetal acceleration (or acceleration oriented along the radius at a radial distance R from the center of the uniformly-rotating disk) is

$$a_c = \omega^2 R. \quad (2)$$

A mass m in such uniform circular motion is subject to a centripetal force

$$F_c = ma_c = m\omega^2 R \quad (3)$$

which is directed inward along the radius to the center of rotation. If the mass is held fixed at this radius, the device causing rotation supplies this force; this is the origin of the static pressure in liquid columns discussed below. If the mass is placed on top of a trap-door above a radially-oriented tube, and the trap-door opened, the inertia of the mass will cause it to accelerate down the tube; this is the basis for driving fluids radially outward on a rotating disk.

Rotation may create a static pressure in a non-flowing fluid. Assume a column of liquid extending from an inner radius R_0. The tube may be along the radius or inclined at an angle to the radius. Let the pressure at position R_0 be defined as P_0, which is for example atmospheric pressure. The excess pressure due to rotation of the liquid at Position R such that $R_0 < R$ is found by integrating the centripetal force per unit area for liquid of density p from position R_0 to R:

$$P - P_0 = \int \rho a_c = \rho\omega^2/2 \times (R^2 - R_0^2) \quad (4)$$

If the tube is filled, extending from the center, then this pressure is

$$P - P_0 = (2.834 \times 10^{-4}) \rho f^2 R^2 \quad (5)$$

in pounds per square inch (psi) where R=radial position in cm, ρ=density in gm/cm^3, and f=frequency in revolutions/sec. Thus, the pressure (or the amount of centripetal force on a fluid) varies directly with the density of the fluid, and as the square of the radial position from the center of rotation as well as the square of the frequency of rotation.

been justified through comparison with numerical solutions. It consists of this: the negative terms on the left-hand-side almost entirely cancel the positive term. Then the right-hand-side can be set to 0 and a solution can be made to the resultant equation for the "terminal velocity" at position R, u_0

$$(\omega^2 (R+L/2) / 2) - (8\mu / \rho a^2)u_0 - 2(u_0^2 / L) = 0 \quad (11)$$

This is a quadratic equation which has the solution

$$u_0 = -(B + \sqrt{B^2 + 4AC}) / 2A \quad (12)$$

with

$$A = L/2$$
$$B = 8\mu / \rho a^2 \quad (13)$$
$$C = (\omega^2 (R+L/2) / 2)$$

In conventional units these become A=2/L, B=320μ /ρD^2 and C=(19.74)f^2(2R+L) with u_0 = fluid velocity in cm/sec; L=droplet length in cm; μ = viscosity in poise; ρ=fluid density in gm/cm^3; D = 2a = tube diameter in cm; and R = radial position of the fluid droplet in cm. As described, this expression gives the approximate velocity of a droplet of fluid in a tubular channel, the volume of the droplet resulting in droplet length being shorter than the channel length. This estimate assumes both viscous and non-viscous losses. The velocity of a fluid droplet will increase with increasing density and droplet volume (length), and decrease with increased viscosity. The velocity will increase with increased channel diameter, rotational velocity, and radial position.

Fluid flow velocity in a filled channel connecting a full chamber at position R_0 and receiving reservoir at position R_1 is calculated by defining L in equation (11) and subsequent equations as the channel length, L= R_1 - R_0. Then equation (13) with the definitions following equation (13) are used to calculate the flow velocity in the filled chamber as a function of radius.

The rate of fluid-flow is the product of velocity and channel area:

$$Q = u_0 \pi a^2 = u_0 \pi D^2 / 4 \quad (14)$$

where Q = flow in mL/sec; u_0 = velocity in cm/sec (calculated from equations 12 and 13); and D = tube diameter in cm.

The time required to transfer a volume V from a reservoir to a receptacle through a tube or channel of length L depends on whether V is such that the tube is filled (length of a "droplet" of volume V in the tube would be longer than the tube itself) or unfilled by volume V. In the former case, this time is approximately the volume V of the fluid divided by the rate of flow Q; in the latter case it is approximately this calculated time

(10μm, 100μm, or 1,000μm), and rotation frequency (100, 1,000 or 10,000 rpm). (As above, for tubes with a non-radial orientation of 45°, the velocity drops by a factor of 30%). Droplet velocities shown in Figure 3A were calculated by Equation 3 and flow rates determined using Equation 4.

In Figures 5A, 5B and 5C, the time required to transfer 1, 10, and 100 μL droplets, respectively, through a 5cm tube is shown. The tube connects two radially oriented reservoirs as illustrated in Figure 5D. Transfer times are a function of radial position of the tube (o-30cm), tube diameter (10μm, 100μm, or 1,000μm), and rotation frequency (100, 1,000 or 10,000 rpm). The curves shown in Figures 5A, 5B and 5C were calculated using Equation 15.

Taken together, these formulate and graphs describe the interrelationship of disk radii and rotation speeds, channel lengths and diameters, and fluid properties such as viscosity and density in determining fluid velocities and flow rates on the disk. The assumptions behind these derivations include viscous losses due to Poiseuille (non-turbulent) flow, with the addition of losses due to non-uniform flow of droplets and at tube inlet and outlet ports. These formulae and graphs provide lower limits for velocities and flow rates. Fluid velocities can range from less than 1cm/sec to more than 1,000cm/sec, and fluid flow rates from less than 1pL/sec to tens of mL/sec for rotation rates ranging from 1 to 30,000 rpm. By combining channel diameters and positions on the disk, it is possible to carry out fluid transfer over a wide range of time scales, from milliseconds to hours and tens of hours for various processes.

Disk Coatings and Composition

Microplatforms such as disks and the components comprising such platforms are advantageously provided having a variety of composition and surface coatings appropriate for a particular application among the wide range of applications disclosed herein. Disk composition will be a function of structural requirements, manufacturing processes, and reagent compatibility/chemical resistance properties. Specifically, disks are provided that are made from inorganic crystalline or amorphous materials, e.g. silicon, silica, quartz, metals, or from organic materials such as plastics, for example, poly(methyl methacrylate) (PMMA), acetonitrile-butadiene-styrene (ABS), polycarbonate, polyethylene, polystyrene, , polyolefins, polypropylene and metallocene. These may be used with unmodified or modified surfaces as described below.

One important structural consideration in the fabrication of the microsystems disks of the invention is mechanical failure due to stress during use. Failure mechanisms for disks rotated at high velocities include fracture, which can arise as the result of

its oxides (essentially silica) are chemically attacked only by some peroxides (such as a mixture of hydrogen peroxide plus sulfuric acid), hydroxides (such as KOH), hydrofluoric acid (HF), either alone or in combination with alkali-based nitrates, and various perfluorinated solvents (like SF_6) *see* Iler, 1979, *The Chemistry of Silica*, Wiley & Sons: New York; *Properties of Silicon*, Xth ed., INSPEC:, London, 1988). Silicon-based substrates are chemically inert to aliphatic and aromatic hydrocarbons (such as tetrahydrofuran, toluene, and the like), and are substantially inert when exposed to water and neutral aqueous solutions.

A wide variety of polymer-based (plastics) substrates are suitable for fabricating microsystems platforms of the invention. The most chemically-resistant polymer, poly(tetrafluoroethylene; PTFE), is not melt-processible but may be easily machined. PTFE is virtually chemically inert and can be used in most applications utilizing strong acids, bases, alkalis, halogenated solvents, or other strong chemical reagents. Other fluoropolymers (such as FEP, PFA) are more easily processed than PTFE and retain most of PTFE's chemical resistance. More easily-processed materials may be chosen for selective resistance: for example, although polyimides are highly resistant to alcohols, alkalis, aliphatic hydrocarbons, and bases (*e.g.*, NaOH), their resistance to partially-halogenated solvents (*e.g.* dichlorobenzene) is poor. Poly (vinyl chloride) is strongly resistant to oxidizing acids and aliphatic hydrocarbons, while its resistance to aromatic compounds is poor. In addition, many materials that are not highly-resistant to concentrated applications of certain chemicals provide sufficient resistance to dilute solutions or provide sufficient resistance for single-use devices (e.g., polyamides and polyimides may be used with dilute solutions of certain acids such as acetic acid and hydrochloric acid). Most polymeric materials are resistant to water.

Specific chemical/polymer combinations include: formamide, lutidine, and acetonitrile with non-aromatic, non-polar polymers (polypropylene, polyethylene); dichloromethane with polycarbonates and aromatic polymers (polystyrene); ethanolamine and dimethyl sulfoxide with aliphatic and non-aromatic polymers (poly(methyl methacrylates), polyimides, polyamides). Fluoropolymers are resistant to all of the above chemical agents. Other solvents and reagents of interest, including pyridine, tetrazole, trichloracetic acid, iodine, acetic anhydride, N-methylpyrrolidine, N,N-diethylpropylethylamine and piperidine, are suitable for use with fluoropolymers and some solvent resistant polymers, such as PVC (*Encyclopedia of Polymer Science*

energies through the formation of surface complexes, for example, hydroxyl-rich surfaces for increased hydrophilicity, or perfluorinated surfaces for increased hydrophobicity. Surface graft polymerization is a technique used to graft polymers or oligomers with the desired surface properties to a substrate polymer chosen for its bulk processability and manufacturing properties, such as a plastic. Commercial methods for initiating graft polymerization include gamma radiation, laser radiation, thermal or mechanical processing, photochemical processes, plasma, and wet chemical processes (further discussed in *Encyclopedia of Polymer Science and Technology*, 2nd ed., (Supplement), Wiley & Sons: New York, 1989, pp 675-689). Chemical modification of polymer surfaces (and appropriate polymers) includes oxidations (polyethylenes), reductions (fluoropolymers), sulfonations, dehydrohalogenations (dehydrofluorination of poly (vinylidene fluoride), and hydrolyses. While the chemical nature of the surface is altered through chemical modification, mechanical properties, durability and chemical resistance are primarily a function of the substrate plastic. For example, surface grafting of poly(ethylene glycol) (PEG) onto polyethylene yields a surface that is both hydrophilic (unlike polyethylene) and resistant to water (PEG is itself soluble in water, while polyethylene is not). Finally, silation of organic polymer surfaces can also be performed, providing a wide variety of surface energy/chemistry combinations.

Embodiments comprising thin film disks are provided, comprising "layers" of microsystems disks stacked on a solid support, are useful for sequential assay with conservation of the disk and efficient and inexpensive use of the microsystem-comprising disks as consumables. An illustration of such disks are shown in Figure 17L. Such disks are capable of being uniquely identified, for example, by imprinting a barcode directly on the disk.

Particular examples of disks fabricated for a variety of applications is provided below in the Examples.

Disk-Related Devices and Elements

Microsystems platforms (microplatforms) of the invention are provided with a multiplicity of on-board components, either fabricated directly onto the disk, or placed on the disk as prefabricated modules. In addition to be integral components of the disk,

constituents. The electrostatic composition of the sieving materials may be inherent to the material or bestowed upon it by virtue of a charge delivered to the material through an electronic circuit. The materials captured by the filter material can be irreversibly bound or can be selectively eluted for further processing by adjusting the composition and ionic strength of buffers or, in the case of an electronically regulated material, by modulating the electronic state of the material.

In yet other embodiments of the filters of the microsystem platforms of this invention, specific components of a sample can be retained in a section, microchannel or reservoir of a disk of the invention by interaction with specific proteins, peptides, antibodies or fragments thereof derivatized to be retained within the surface of a component of the disk. Materials captured by such specific binding can be eluted from the surface of the disk and transferred to a collection reservoir by treatment with appropriately-chosen ionic strength buffers, using conventional methods developed for immunological or chromatographic techniques.

The invention also provides compartments defined by sections of a microchannel or by a chamber or reservoir wherein the inlet and outlet ports of the chamber are delimited by a filtering apparatus. In certain embodiments, the chamber thus defined contains a reagent such as a bead and particularly a bead coated with a compound such as an antibody having an affinity for a contaminant, unused reagent, reaction side-product or other compound unwanted in a final product. In the use of disks comprising such a filter-limited chamber, a fluid containing a mixture of wanted and unwanted compounds is moved through the filter chamber by centripetal force of the rotating disk. The unwanted compounds are thus bound by the affinity material, and the desired compounds flushed free of the chamber by fluid flow motivated by centripetal force. Alternatively, the desired compound may be retained in such a filter-limited chamber, and the unwanted compounds flushed away. In these embodiments, egress from the chamber, for example by the opening of a valve, is provided.

3. Mixers

A variety of mixing elements are advantageously included in embodiments of the microsystems disks of the invention that require mixing of components in a reaction chamber upon addition from a reagent reservoir. Static mixers can be incorporated into fluid handling structures of the disk by applying a textured surface to the microchannels

from the center inlet port to the outlet with no applied voltage. With a voltage applied the piezo element presses down on the arch center causing the ends to lift, blocking the center inlet and allowing fluid to flow from the peripheral inlet. In other, two-way embodiments, fluid flows with no applied voltage and is restrained upon the application of voltage. In another embodiment of a two-way valve, fluid is restrained in the absence of an applied voltage and is allowed to flow upon application of a voltage. In any of these embodiments the piezo stack can be perpendicular to the plane of rotation, oblique to the plane of rotation, or held within the plane of rotation.

In another embodiment, fluid control is effected using a pneumatically-actuated microvalve wherein a fluid channel is etched in one layer of material that has a raised valve seat at the point of control (a schematic diagram of this type of valve is shown in Figure 7). Into another layer, a corresponding hole is drilled, preferably by a laser to achieve a hole with a sufficiently small diameter, thereby providing pneumatic access. Onto that second structure a layer of silicone rubber or other flexible material is spun-deposited. These structures are then bonded together. Fluid movement is interrupted by the application of air pressure which presses the flexible membrane down onto the raised valve seat. This type of valve has been described by Veider *et al.* (1995, Eurosensors IX, pp. 284-286, Stockholm, Sweden, June 25-29). Measurements made by Veider *et al.* have shown that a similar valve closes completely with the application of 30 KPa of pressure over the fluid inlet pressure. This value corresponds to 207 psig, and can be adjusted by changing the diameter of the pneumatic orifice and the thickness of the membrane layer. Pneumatic pressure is applied to the disk to activate such valves as shown schematically in Figure 8.

Pneumatic actuation can also be embodied by a micromachined gas valve that utilizes a bimetallic actuator mechanism, as shown in Figure 9. The valve consists of a diaphragm actuator that mates to the valve body. The actuator can contain integral resistive elements that heat upon application of a voltage, causing a deflection in the diaphragm. This deflection causes a central structure in the actuator to impinge upon the valve opening, thus regulating the flow of fluid through the opening. These valves allow proportional control based on voltage input, typically 0 - 15 V DC. These types of valve are commercially available (Redwood Microsystems, Menlo Park, CA; ICSensors, Milpitas, CA).

structure equilibrates. A common example of this phenomenon is contraction of polyolefin (used in heat shrink tubing or wrap), the polyolefin structure of which is stable at room temperature. Upon heating to 135°C, however, the structure contracts. Examples of PR valve polymers include but are not limited to polyolefins, polystyrenes, polyurethanes, poly(vinyl chloride) and certain fluoropolymers.

One way to manufacture a PR valve is to place a polymer sheet or laminate over a channel requiring the valve (as shown in Figure 11). A cylindrical valve is then cold-stamped in such a way as to block the microchannel. The valve is actuated by the application of localized heat, for example, by a laser or by contact with a resistive heating element. The valve then contracts and fluid flow is enabled.

A further type of microvalve useful in the disks of the invention is a single use valve, illustrated herein by a capillary microvalve (disclosed in U.S. Provisional Application Serial No. 60/00x,xxx, filed August x, 1996 and incorporated by reference herein). This type of microvalve is based on the use of rotationally-induced fluid pressure to overcome capillary forces. Fluids which completely or partially wet the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.) which contain them experience a resistance to flow when moving from a microchannel of narrow cross-section to one of larger cross-section, while those fluids which do not wet these materials resist flowing from microchannels (or reservoirs, reaction chambers, detection chambers, etc.) of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two microchannels (or reservoirs, reaction chambers, detection chambers, etc., or combinations thereof), the surface tension of the fluid, and the contact angle of the fluid on the material of the microchannels (or reservoirs, reaction chambers, detection chambers, etc.) . Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional dimension results in microchannels of dimension less than 500μm exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the microsystems platform of the invention, "valve" are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency, with the radial position and with the extent of the

to external databases and modems for remote data transfer. Specifically, controllers can be integrated into optical read systems in order to retrieve information contained on the disk, and to write information generated by the analytic systems on the disk to optical data storage sections integral to the disk. In these embodiments it will be understood that both read and write functions are performed on the surface of the disk opposite to the surface comprising the microsystems components disclosed herein..

Information (*i.e.*, both instructions and data, collectively termed "informatics") pertaining to the control of any particular microanalytic system on the disk can be stored on the disk itself or externally, most advantageously by the microprocessor and/or memory of the disk device of the invention, or in a computer connected to the device. The information is used by the controller to control the timing and open/closed state of microvalves on the disk, to determine optimal disk rotational velocity, to control heating and cooling elements on the disk, to monitor detection systems, to integrate data generated by the disk and to implement logic structures based on the data collected.

7. Power Supply

The electrical requirements of systems contained on a disk can be delivered to the disk through brushes that impinge upon connections integral to the disk. Alternatively, an electrical connection can be made through the contact point between the microplatform and the rotational spindle or hub connecting the disk to the rotational motivating means. A battery can be integrated into the disk to provide an on-board electrical supply. Batteries can also be used to power the device used to manipulate the disk. Batteries used with the invention can be rechargeable such as a cadmium or lithium ion cell, or conventional lead-acid or alkaline cell.

Power delivered to the disk can be AC or DC. While electrical requirements are determined by the particular assay system embodied on the disk, voltage can range from microvolts through megavolts, more preferably millivolts through kilovolts. Current can range from microamps to amperes. Electrical supply can be for component operation or can be used to control and direct on-disk electronics.

Alternatively, inductive current can be generated on the disk by virtue of its rotation, wherein current is provided by an induction loop or by electrical brushes. Such current can be used to power devices on the disk.

embodiments, the optical component preceding the detector can include a dispersive element to permit spectral resolution. Fluorescence excitation can also be increased through multiple reflections from surfaces in the device whenever noise does not scale with path length in the same way as with signal.

Another type of fluorescence detection configuration is shown in Figure 12B. Light of both the fluorescence excitation wavelength and the emitted light wavelength are guided through one face of the device. An angle of 90 degrees is used to separate the excitation and collection optical trains. It is also possible to use other angles, including 0 degrees, whereby the excitation and emitted light travels colinearly. As long as the source light can be distinguished from the fluorescence signal, any optical geometry can be used. Optical windows suitable for spectroscopic measurement and transparent to the wavelengths used are included at appropriate positions (*i.e.*, in "read" reservoir embodiments of detecting chambers) on the disk. The use of this type of fluorescence in macroscopic systems has been disclosed by Haab *et al.* (1995, *Anal. Chem.* 67: 3253-3260).

2. Absorbance Detection

Absorbance measurements can be used to detect any analyte that changes the intensity of transmitted light by specifically absorbing energy (direct absorbance) or by changing the absorbance of another component in the system (indirect absorbance). Optical path geometry is designed to ensure that the absorbance detector is focused on a light path receiving the maximum amount of transmitted light from the illuminated sample. Both the light source and the detector can be positioned external to the disk, adjacent to the disk and moved in synchrony with it, or integral to the disk itself. The sample chamber on the disk can constitute a cuvette that is illuminated and transmitted light detected in a single pass or in multiple passes, particularly when used with a stroboscopic light signal that illuminates the detection chamber t a frequency equal to the frequency of rotation or multiples thereof. Alternatively, the sample chamber can be a planar waveguide, wherein the analyte interacts on the face of the waveguide and light absorbance is the result of attenuated total internal reflection (*i.e.*, the analyte reduces the intensity source light if the analyte is sequestered at the surface of the sample chamber, using, for example, specific binding to a compound embedded or attached to

the presence of the analyte as antibody from more than one particle bind to the analyte. When the disk is spun after this interaction occurs, sample chambers containing analyte will be less turbid that sample chambers not containing analyte. This system can be calibrated with standard amounts of analyte to provide a gauge of analyte concentration related to the turbidity of the sample under a set of standardized conditions.

Other types of light scattering detection methods are provided for use with the microsystems platforms and devices of the invention. Monochromatic light from a light source, advantageously a laser light source, is directed across the cross-sectional area of a flow channel on the disk. Light scattered by particles in a sample, such as cells, is collected at several angles over the illuminated portion of the channel (*see* Rosenzweig *et al.*, 1994, *Anal. Chem.* 66: 1771-1776). Data reduction is optimally programmed directly into the device based on standards such as appropriately-sized beads to relate the signal into interpretable results. Using a calibrated set of such beads, fine discrimination between particles of different sizes can be obtained. Another application for this system is flow cytometry, cell counting, cell sorting and cellular biological analysis and testing, including chemotherapeutic sensitivity and toxicology.

b. Electrochemical Detection Methods

Electrochemical detection requires contact between the sensor element and the sample, or between sensor elements and a material such as a gas in equilibrium with the sample. In the case of direct contact between sample and detector, the electrode system is built directly onto the disk, attached to the disk before rotation or moved into contact with the disk after it has stopped rotating. Detectors constructed using a gas vapor to encode information about the sample can be made with the detector external to the disk provided the gas vapor is configured to contact both the sample chamber and the detector. Electrochemical detectors interfaced with the disk include potentiometric, voltammetric and amperimetric devices, and can include any electrochemical transducer compatible with the materials used to construct the microsystem disk.

1. Electric Potential Measurement

One type of electrochemical detection means useful with the microsystems platforms of the invention is an electrical potential measurement system. Such a system

comprising up to 16 lines of an electrode fabricated in a chamber by photolithography with dimensions resulting in each line being 100μm wide with 50 μm between lines. (*see* Aoki *et al.*, 1992, *Anal. Chem.* 62: 2206). In this embodiment, a volume of fluid containing a substance of interest is directed to the chamber. Within the chamber each electrode is set a different potential so that 16 separate channels of electrochemical measurement may be made. Additionally, each electrode potential can be swept stepwise by a function generator. This protocol yields information pertaining to redox potential as well as redox current of the substances. This type of analysis also allows identification of molecules *via* voltammogram.

c. Physical Methods

Physical detection methods are also provided for use with the disks of the invention. For example, the disk can be used as a viscometer. Microchannels containing fluid to be tested advantageously contain a bead inserted on the disk. The motion of the bead through the fluid is analyzed and converted into viscosity data based on standards developed and stored in microprocessor memory. (*see* Linliu *et al.*, 1994, *Rev.Sci. Instrum.* 65: 3824-28).

Another embodiment is a capacitive pressure sensor (*see* Esashi *et al.*, 1992, *Proc. Micro Electro Mechanical Systems* 11: 43). In this embodiment, silicon and glass substrates are anodically bonded with hermetically sealed reference cavities. Pressure may be detected by the capacitance change between the silicon diaphragm and an aluminum electrode formed on the glass. A capacitance-to-frequency converter output of a CMOS circuit can be integrated on the silicon substrate or contained in controlling electronics off the disk.

By judicious placement of pressure sensors, the pressure due to centrifugation can be determined at any position on the disk. In conjunction with the microchannel diameter information and the pattern of orientation of the channels on the disk, pressure data can be used to determine flow rates at a particular rotational speed. This information can then be used by the microprocessor to adjust disk rotational speed to control fluid movement on the disk.

Surface acoustic wave (SAW) devices are also provided as components of the

Methods and Uses

Because of its flexibility, the invention offers a myriad of possible applications and embodiments. Certain features will be common to most embodiments, however. These features include sample collection; sample application to disk, incorporating tests of adequacy at the time of sample application; a variety of specific assays performed on the disk; data collection, processing and analysis; data transmission and storage, either to memory, to a section of the disk, or to a remote station using communications software; data output to the user (including printing and screen display); and sample disk disposal (including, if necessary, disk sterilization).

Sample or analyte is collected using means appropriate for the particular sample. Blood, for example, is collected in vacuum tubes in a hospital or laboratory setting, and using a lancet for home or consumer use. Urine can be collected into a sterile container and applied to the disk using conventional liquid-transfer technology. Saliva is preferably applied to the disk diluted with a small volume of a solution of distilled water, mild detergent and sugar flavoring. This solution can be provided as a mouthwash/gargle for detecting antigens, biological secretions and microorganisms. Alternatively, a small sack made of a fishnet polymer material containing the detergent formulation and a chewable resin can be chewed by a user to promote salivation, and then removed from the mouth and saliva recovered and applied conventionally. Amniotic fluid and cerebrospinal fluid are, of necessity, collected using accepted medical techniques by qualified personnel.

Environmental and industrial samples are collected from ground water or factory effluent into containers produced to avoid leaching contaminants in the sample. Soil samples are collected and mixed with a solvent designed to dissolve the analyte of interest. Industrial applications, such as pyrogen screening, are accomplished using specially-designed sample ports.

Sample or analyte is loaded onto the disk by the user. Sample is optimally loaded onto the disk at a position proximal to the center of rotation, thereby permitting the greatest amount of centripetal force to be applied to the sample, and providing the most extensive path across the surface of the disk, to maximize the number, length or arrangement of fluid-handling components available to interact with the sample.

may be contained within the disk or on disk surface. Some on-disk devices have been described above in detail; additionally, the disk may contain electronic circuitry, including microprocessors for coordination of disk functions, and devices for communication with the disk manipulation device or other devices. The disk optimally comprises detectors and sensors, or components of these devices and energy sources for various detection schemes (such as electric power supplies for electrochemical systems, electromagnetic radiation sources for spectroscopic systems), or materials, such as optically-transparent materials, that facilitate operation of and data generation using such detectors and sensors; actuators, including mechanical, electrical, and electromagnetic devices for controlling fluid movement on the disk, including valves, channels, and other fluid compartments; communications and data handling devices, mediating communications between the disk and the player/reader device, using electromagnetic (laser, infra-red, radiofrequency, microwave), electrical, or other means; circuitry designed for controlling procedures and processes on the disk, including systems diagnostics, assays protocols and analysis of assay data. These are provided in the form of ASICs or ROM which are programmed only at the point-of-manufacture; FPGA's EPROM, flash memory (UV-erasable EPROM), or programmable IC arrays, or similar arrays programmable by the user through the platform manipulation device or other device. Also included in the components of the invention are CPU and microprocessor units and associated RAM operating with an assembler language or high-level language programmable through disk communications, and components for mediating communication with other devices, including facsimile/modem communications with remote display or data analysis systems.

Off-disk devices comprise the microplatform micromanipulating device itself and other devices which can access information, write information, or initiate processes on the disk. Figure 15 illustrates the categories of devices and sub-devices which are part of the micromanipulation device , and indicates how there components interact. "Interaction" is used herein to mean the exchange of "data" between the disk and device, or among the components of the device itself . The relationship between these components is here described, followed by detailed examples of the components.

These include the mechanical drive and circuitry for rotation monitoring and

devices, and printers, plotters, and graphics devices are provided as components of the microplatform micromanipulating devices of the invention. Communication and telecommunications are provided through standard hard-wired interfaces (such as RS-232, IEE-488M SCSI bus), infra-red and optical communications, short-or long-range telecommunications ("cellular" telecommunications radio-frequency), and internal or external modem for manual or automated telephone communications.

Disk information comprises both software written to the disk to facilitate operation of the microsystem assays constructed thereupon, and assay data generated during use of the microsystem by the user. Disk information includes material written to the disk (as optically encoded data) and information inherent to the disk (*e.g.*, the current status of a valve, which can be accessed through magnetic pickup or through the reflective properties of the coating material at the valve-position) Data written to the disk may include but is not limited to the audio/video/test and machine format information (*e.g.*, binary, binhex, assembler language). This data includes system control data used for initiation of control programs to spin the disk, or perform assays, information on disk configuration, disk identity, uses, analysis protocols and programming, protocols descriptions, diagnostic programs and test results, point-of-use information, analysis results data, and background information. Acquired data information can be stored as analog or digital and can be raw data, processed data or a combination of both.

System control data include synchronization data to enable the micromanipulation device to function at the correct angular velocity/velocities and accelerations and data relating to physical parameters of disk. Disk configuration and compatibility data include data regarding the type of disk (configuration of on-disk devices, valves, and reagent, reaction and detection chambers) used to determine the applicability of desired testing protocols; this data provides a functional identity of the type of disk and capabilities of the disk. It can be also form part of an interactive feedback system for checking microsystem platform components prior to initiation of an assay on the disk. Disk identify and serial numbers are provided encoded on each disk to enable exact identification of a disk by fabrication date, disk type and uses, which data are encoded by the manufacturer, and user information, which is written to the disk

and data output blocks. The particular acquisition process here involves using an encoded signal on the disk—for example, an optical signal associated with a detection chamber passing the detector—to gate acquisition of data. In this way, data is acquired for a specific time when detection chambers are in proximity to the detector. It is also possible to continuously take data and use features in that data— for example, the shape of the signal as a function of time, which might look like a square wave for an array of windows on an otherwise opaque disk– -to determine what parts of the data are useful for analysis. Data analysis could include non-linear least-squares fitting, linear regression of data as function of time, or end-point analysis (data at an end-point time for a reaction), as well as other methods. Data output may be in the form of "yes/no" answers to the user interface, numeric data, and storage to internal or external storage media.

All component parts of this program need not be contained on the disk. For example, the program can be resident in the computer and designed to read the disk itself to obtain the rotation velocity profiles necessary for using the disk. All other aspects of the program—such as when and how to read and analyze data—can be part of a dedicated program or read from other media.

Analysis/test protocol data are descriptions of tests and analyses which can be performed with a disk. These data can be a simple as a title given the disk, or can contain a detailed description of disk use, data analysis and handling, including test protocols and data analysis protocols. Analysis/test protocol programming is provided that can be used as systems-specified subroutines in more general software schemes, or can be fed into programmable logic so that the device can perform the desired analyses. Analysis/protocol descriptions are provided, as audio, video, text or other descriptions of analytic processes performed on disk, including background information, conditions for valid use, precautions, and other aspects.

Encryption and verification data/programming is provided to ensure the security of the programming and data generated in the analyses performed by the disk. Encryption/de-encryption routines are used to restricted access to data contained on the disk. Such routines also used in medical diagnostic applications.

System self-diagnostics are also provided. System diagnostics include diagnostic

purposes. Disk data storage media include optical media, utilizing reflecting/non-reflecting flats and pits on a surface, using technology adapted from audio CD, CD-ROM, and "Laserdisc" technology, and barcodes. Magnetooptic and magnetic media are also within the scope of this aspect of the invention, using conventional computer magnetic storage media. Electronic data storage means are also provided, using the status of internal arrays of electronic components (FPGAs, PLAs, EPROM, ROM, ASICs, IC networks) for information handling. Chemical recording means, including simple chromatographic staining of a detector section or chamber of the device, is also disclosed to provide a simple visual record of a test result. This simple chemical recording means provides an avenue to at-home diagnostic without the need for an expensive device more sophisticated in capabilities than required to determine an assay amenable to simply the presence or absence of chemical markers.

Software and Communications

Software providing the information and instruction set for microsystem performance, quality control, data acquisition, handling and processing , and communications is included within the scope of this invention. For the purposes of this invention, such software is referred to as "machine language instructions." Control and analysis software is advantageously provided in high-level languages such as C/C ++, Visual Basic, FORTRAN or Pascal. Drivers are provided for interface boards (either internal to the device or to a host computer interfaced with the device) which translates instructions on the host computer's bus into micromanipulator commands. Additionally, drivers for experiment-control software such as LabView may be created, again using conventional, industry-standard interface protocols. These applications are most preferably capable of being run on a number of popular computer platforms, including UNIX/Linux, X-windows, Macintosh, SGI, etc. Control and analysis can also be performed using dedicated chipsets and circuitry, ROM, and EPROM. For example, test validity can be insured (at least in part) through the use of ROM-based test procedures, in which all programming is performed at the point-of-manufacture without possibility of end-user corruption. Separate application software can also be developed so that data from a disk-player can be analyzed on non-controller platforms, using available

provided on-device. In either case, a user-interface through keyboard, touchpad and/or display components of the device is provided.

Applications software is provided in read-only or re-programmable software formats. Included in this component of the fluidics micromanipulation apparatus of the invention is software that can be read from standard computer data storage medium. Examples include medical or analytic diagnostic programs reliant on integrated data-bases which are contained within disk or device memory, or that can be accessed from networked workstations, or access on-line services, such as a newsletter and news services, and software for the production and analysis of images, including pattern recognition, statistical analysis software, etc.

Integration of control and applications software can be made through the use of either a unique operating system developed for the disk and micromanipulator of the invention, or by adaptation of existing OS. Optimally, the OS uses authoring software to combine text, graphics, video and audio into an easy to use, "point and click" system. Such as OS could also provide an object-oriented environment or facsimile thereof (*e.g.*, LabView-based systems) for customizing programming by sophisticated users, as well as providing for the development of additional software by the disk reader/player manufacturer or independent software developers.

The OS can also be chosen to allow design of disks and disk-based assays. Mechanical design, including simulation of rotational dynamics and stability and fluid flow simulation are advantageously encompassed in a disk design software package.

Communications aspects of the invention include hardware and software embodiments relating to data input and output from a user or to remote control and analysis sites. Hard-wired communications features include high-speed data-, video- or image-transmission and communication through local busses (*e.g.*, a VGA bus for video signals) and conventional hard-wired interfaces (*e.g.*, RS-232, IEEE-488, SCSI bus), Ethernet connections, Appletalk, and various local area networks (LANs). Telecommunications devices include cellular transceivers for short-range communications, radio-frequency and micro-wave transceivers for long-range communications, and internal or external modem for manual or automated telephone communications. Video in/out ports, analog out-lines for data transmission, input jacks

same procedure using a single disk. Device actuation is optimally obtained with the pressing of a single button. These processor(s) and data processors(s) of this type of device comprise circuitry and chipware designed to process analysis data (assay processor) and encoded data (data processor). Information from these processors can be available for output to the user on a front-panel or video display and can also be used internally to ensure correct operating conditions for the assay. This internal information processing can include the results of systems diagnostic tests to insure disk identity and test type compatibility; the presence of reagent and sample as determined through light absorption through a detector port scanning reagent and sample reservoirs; the presence of contamination detected before testing begins, and the results of self-diagnostics on external detectors and actuators. These results are used by the system controller to determine whether the requested test can be performed.

After loading and activation, analysis results can be stored internally in electronic memory or encoded upon the disk. The results of these analyses and procedures are then routed to the front-panel display (flat-panel LCD, etc.) using appropriate video drivers. Processed assay data can also be routed to one of many standard digital I/O systems including RS-232, RS-232C, IEEE-488, and other systems familiar from digital I/O and interface. Similarly, encoded disk data can be routed to the audio/visual display. Raw analog signals can also be switched to one or more external jacks for off-device storage or processing.

An embodiment of the least technically sophisticated device is a portable unit no larger than a portable audio CD player consisting of disk-drive, controllers and selectors for programmable or pre-programmed angular acceleration/deceleration profiles for a limited number of procedures. Such a device is advantageous for on-site toxic-chemical/contamination testing. Analyte to be tested is introduced to the disk, which is inserted into the player and the appropriate program chosen. Analysis results are stored on the disk, to be later read-out by a larger player/reader unit, and/or displayed immediately to the user. Results can also be stored as the inherent state of an indicator (positive/negative status of litmus paper in different cuvettes, for example), with no other data collection or analysis performed by the device. This data would be accessed by a larger player/reader or by other means outside the field-work environment.

integrated system is provided with extensive analysis software and background databases and information. Disk-storage cassettes of carousals are also an advantageous feature of such system. An integrated system of this type is useful in a large, analytical laboratory setting.

A self-contained system is useful for applications in isolated environments. Examples include devices used in remote or hostile setting, such as air, water and soil testing devices used in the Arctic for environmental purposes, or for use on the battlefield for toxic chemical detection.

The microsystem platforms provided by the invention are also useful for preparing samples for other analytical instruments, such as mass-spectrometers, gas chromatographs , high pressure liquid chromatographs, liquid chromatographs, capillary electrophoresis, inductively-coupled plasma spectroscopy, and X-ray absorption fine-structure. In some application, the final product is removed from the disk to be analyzed.

Samples can be pre-concentrated and purified on the device by incorporating aqueous two-phase separation systems. This can be done, for example, by mixing two phases which separate from each other based on thermodynamic differences like polyethylene glycol (PEG) and dextrans; biopolymers are usefully separated using this method. Alternatively, environmental tests such as colorimetric analysis can be enhanced by incorporating cloud-point separations to concentrate and enhance optical signals. In addition, small scale counter-current chromatography can be performed on the device (*see*, Foucault, 1991, *Anal.* Chem. 63: PAGE). Centripetal force on the disk can be used to force different density fluids to flow against each other, resulting in separation of components along a density gradient to develop the chromatogram.

Applications and Uses

The microsystem platforms and micromanipulating devices that make up the fluidics micromanipulation apparatus of the invention have a wide variety of microsynthetic and microanalytic applications, due to the flexibility of the design, wherein fluids are motivated on the platform by centripetal force that arises when the platform is rotated. What follows is a short, representative sample of the types of

sandwich.

An example of a disk adapted for performing such an immunoassay is shown in Figure 17Q. In this embodiment, the secondary antibody is linked to alkaline phosphate (AP). The presence and amount of AP activity is determined by monitoring the conversion of one of the following exemplary substrates by the enzyme colorimetrically: B-naphthyl phosphate converts to an insoluble azo dye in the presence of a diazonium salt; 5-bromo-4-chloro-3-indolyl phosphate is converted to 5,5'-dibromo-4-,4'-dichloro indigo in the presence of cupric sulfate; or 4-methylumbelliferyl phosphate is converted to 4-methylumbelliferone, which emits light at 450nm.

In one exemplary embodiment, the reaction chamber comprises an antibody specific for an antigen, where the antibody is immobilized by adsorption of the antibody to the reaction chamber. Contiguous with the reaction chamber is advantageously placed a reagent reservoir containing a second antibody, this antibody being liked to an enzyme such as alkaline phosphate. Sample, which may contain an antigen of interest that is specifically recognized by the above antibodies, is loaded at an inlet port. The disk is spun to first introduce the sample into the reaction chamber containing immobilized antibody, followed by introduction of the second antibody into the reaction chamber after a time sufficient to saturate the immobilized antibody with antigen to the extent the antigen is present in the sample. Alternatively, the sample may be contacted with the second antibody, allowed to interact, then introduced into the reaction chamber. Incubation of the sample with antibody is performed without spinning for about 1 minute. After each incubation, washing buffer from a buffer reservoir is spun into the reaction chamber in order to remove unbound antibody. For alkaline phosphatase assays, solutions of 2mg/mL *o*-dianisidine in water, 1mg/mL *B*-naphthyl phosphate in 50mM boric acid/50mM KCl (pH 9.2) buffer and 100 mM magnesium chloride are delivered to the reaction chamber in the appropriate amounts. The extent of enzyme-linked, secondary antibody binding is evaluated by detection of a purple precipitate using a photodiode or CCD camera.

A disk configured for immunoassay applications is shown in Figure 17R for illustration.

In an alternative embodiment of the immunological assays of the invention, the

additional alternatives, microlithographic and microstamping techniques can be used to prepare the surface or chamber.

In the practice of the invention, a biological or other fluid sample containing the particular cell or cell type of interest is applied to the prepared surface or chamber and allowed in contact with the prepared surface or chamber for a time sufficient to allow specific binding of the cells or cell types to the surface. As contact with the surface may be inhibited by cell settling properties in the volume of the fluid, chambers and surfaces having minimized height transversely through the microsystem platform are preferred.

Non-specific cell binding is minimized or eliminated from the chamber or surface by washing the surface or chamber with a fluid amount sufficient to remove such non-specific binding. Washing is accomplished by simple bulk flow of fluid over the surface or chamber, or by centrifugation.

After washing, cells that remain attached to the surface or chamber are detected and counted. In a preferred embodiment, detection and counting is achieved using fluorescence microscopy. In the practice of the invention, specific dyes can be used to provide a fluorescence signal for any live cells remaining of the disk. The dye can be added directly to the surface or chamber, for example using a membrane-permeant dye, such as acetoxy-methyl ester dyes. Alternatively, specific antibodies can be linked to such dyes. Dyes can be added to the biological fluid comprising the cells prior to introduction onto the microsystem platform, or such dyes can be contacted with the cells *in situ* on the disk. The presence of the cells is detected using a fluorescence detector comprising a light source, a source filter, a dichroic filter or mirror, an emission filter, and a detector such as a photomultiplier tube.

In another example, thin-layer chromatography is accomplished on a microplatform disk comprising 100 pm square cross-section channels radiating outward from the center of the disk. Each channel is filled with separation substrate, which typically contains a binder material (0.1-10%) such as starch, gypsum, polyacrylic acid salts and the like, to provide mechanical strength and stability. (The use of such compounds in conventional TLC applications is discussed in Poole *et al.*, 1994 *Anal. Chem.* 66: 27A). Sorbents are also included in the materials comprising the separation

immunoassay for metabolites, drugs, and other biological and other chemical species; vaccine efficacy monitoring; myeloma or lupus erythematosus monitoring; determination of blood glucose and/or ketone body levels in patients with diabetes; automated cholesterol testing: automated blood drug concentration determination; toxicology; monitoring of electrolytes of** other medically-relevant blood component at a patient's bedside; sepsis/endotoxin monitoring; allergy testing; and thrombus monitoring.

The invention also provides analytical instruments for environmental testing, industrial applications and regulation compliance. Portable, preferably hand-held embodiments, as well as more extensive embodiments, installed as part of an industrial quality control regime, are provided. Applications for these embodiments of the invention include analyte testing, particularly testing for industrial effluents and waste material, to be used for regulatory compliance; and quality control of industrial, most advantageously of human consumable items, particularly pharmaceuticals and specifically endotoxin determinations. Application for testing, mixing and evaluating perfumes and other complex mixtures are also within the scope of the invention.

The invention also provides chemical reaction and synthesis modeling, wherein a reaction scheme or industrial production regime can be tested and evaluated in miniaturized simulations. The invention provides for cost-effective prototyping of potential research, medical and industrial chemical reaction schemes, which can be scaled to macroscopic levels after analysis and optimization using the microsystems platforms of this invention.

A variety of other applications are provided, including microsynthetic methods and forensic applications.

The following Examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

. adapted for particular applications as described in the Figure legends.

EXAMPLE 2

Blood Composition Determination

Blood composition can be determined *via* hematocrit analysis using an analytic microplatform disk prepared as described in Example 1 held within a device comprising a microchannel layer with a number of microchannels as shown in Figure 18. The microchannel layer is 100pm thick and treated with heparin to prevent coagulation during the assay. The blood sample to be analyzed is drawn by capillary action into a channel arranged perpendicular to the direction of rotation, as shown in Figure 18; a number of such channels may be arranged radially on the disk. When all samples to be tested have been drawn into the channels, the disk is spun at a speed of 8000 to 10,000 rpm to effect sedimentation of erythrocytes within the channel. Once centrifugation has been performed for an appropriate time (3 to 5 minutes), the hematocrit of each sample is determined simultaneously by stroboscopic interrogation of each of the channels using a conventional CD laser system in the device described above. When the laser passes the boundary of erythrocytes, the change in light scattering pattern detected by the photodiode detector is converted into a hematocrit value based on a standardized set of light scatter/hematocrit information stored in the internal processor and memory of the device. Alternatively, the raw information is relayed *via* a infrared port or hard-wired interface to a microprocessor for analysis. Such a central microprocessor is on site or in the alternative at a centralized location, such as a nursing station in a hospital or in a medical center connected to the hematocrit determining device by telephone or other dedicated connection. Hematocrit can be determined by untrained individuals (including patients) by the simple application of a blood droplet produced by lancet onto the disk, followed by the simple application of the device and automated hematocrit analysis and data processing on site or transmission to a central location of trained medical personnel. This embodiment of the invention provides for chronic monitoring of patients having hematopoietic proliferative disease (such as leukemia, lymphoma, myeloma, and anemias).

In addition, blood gas can be determined using the above device in combination with a disk having integrated electrodes embedded within the hematocrit channel, or having a separate channel devoted to blood gas determination on the hematocrit disk. Blood oxygenation (PO_2) is determined by a Clark-type electrode consisting of a thin Cr-Au cathode and an Ag-AgCl wire anode. The amount of carbon dioxide in the blood is determined by a Severing-type electrode using an ISFET (a type of field effect transistor) as a pH monitor. Blood pH is determined with the use of a Si_3N_4 gate ISFET

in platelets toward the center of rotation and proteins toward the periphery. Fraction 1 results in platelets toward the center of rotation and proteins toward the periphery. Fraction 2 yields fractions 3 and 4, consisting of lymphocytes and monocytes toward the center of rotation and erythrocytes and neutrophils toward the center of rotation and monocytes toward the periphery. Fraction 4 yields neutrophils toward the center of rotation and erythrocytes toward the periphery. Thus, fractionation of blood into five isolated components is achieved.

The activity of enzymes in the protein fraction can be determining using immobilized enzymes (Heineman, 1993, *App. Biochem. Biotech.* 41: 87-97). For example, blood-specific enzymes (such as glucose oxidase, alkaline phosphatase, and lactate oxidase) can be immobilized in poly (vinyl alcohol (PVAL). Lactate oxidase is immobilized on platinized graphite electrodes by sandwiching a thin layer of enzyme between two layers of PVAL. The sensor responds to lactate by the electrochemical oxidation of hydrogen peroxide generated by the enzyme-catalyzed oxidation of lactate that diffuses into the network. The current produced is proportional to the concentration of peroxide, which in turn is proportional to the concentration of lactate. This sensor has been shown to be sensitive to lactate concentrations ranging form 1.7-26 uM.

Upon separation, each fraction is interrogated by detection systems to determine the relative components of the fractions. Alternatively, each fraction can be removed from the disk through an outlet port for further study off-device. For example, each fraction can be subjected to simple counting by passing the cells in a thin steam past two electrodes comprising a resistance monitor. As a cell passes through the electrodes a corresponding rise in resistance is monitored and counted. These data are then integrated relative to a standard set of particles distributed according to size to determine the relative number of each cell type in the original sample.

The fractions can be subjected to fluorescent antibody staining specific to each cell type. The cells are held in place by micromachined filters integral to the channels (U.S. Patent No. 5,304,487), stained and washed on the disk. The resulting labeled cells can then be quantified as a function of the degree of fluorescent staining associated with the cells.

EXAMPLE 3

DNA sizing and mutation detection

DNA sizing and detection of specific mutations in DNA at a particular site are carried out using double stranded melting analysis with a disk prepared according to Example 1 and illustrated in Figure 20. A DNA meltometer (as described in co-owned and co-pending U.S. Serial No. 08/218,030, filed March 24, 1994 and incorporated

Nos. 08/375,226, filed January 19, 1995, which is a file wrapper continuation of USSN 08/074,345, filed June 9, 1993 and 08/353,573, filed December 8, 1994, each incorporated by reference in its entirety). Amplification is carried out using one primer labeled with a detectable label such as a fluorescent dye or radioisotope, and the other primer is covalently attached to a molecule that permits immobilization of the primer (e.g., biotin). After amplification (either off-disk or on the disk as described in more detail in Example 4 below), the labeled, biotinylated duplex DNA product fragment is attached to a solid support coated with streptavidin, for example, by movement of the amplification reaction mixture into a channel or compartment on the disk wherein the walls are coated with streptavidin, or by movement of the amplification mixture into a compartment on the disk containing a binding matrix such as Dynal M-280 Dynabeads (polystyrene coated paramagnetic particles of 2.8um in diameter). Standardized size markers are included in the post-amplification compartment in order to provide a reference set of DNA fragments for comparison with the amplification product fragments. In this analysis, a number of different duplex DNA molecules from either a multiplex amplification reaction or a number of separate amplification reactions may be sized simultaneously, each fragment or set of fragments being distinguished from others by use of reaction- or fragment-specific detectable labels, or differences in some other physical property of the fragments. For amplifications performed off-disk, beads attached to the fragment are loaded into a channel on the disk capable of retaining the beads (such as size exclusion, "optical tweezers" or by magnetic attraction). In the latter embodiment, the magnetic retention means (permanent magnets or electromagnets) are either integral to the disk, held on second disk spinning synchronously with the first, or placed on the device so as to immobilize the DNA fragments in the appropriate compartment.

DNA size analysis is also performed essentially as described above, whereby the retained particles are subjected to a thermal denaturing gradient. For a thermal gradient used to denature the bound DNA fragments, a Peltier heat pump, direct laser heating or a resistive element is used to increase the temperature of the binding compartment through the denaturation range by the gradual addition of thermal energy. As above, a flow rate of 10μL/min can be generated in a channel 100μm in diameter, allowing a melting ramp of 30 min. The compartment is also subjected to a flow stream as described above to elute the denatured, labeled stands from the binding/melting chamber. Downstream from the binding/melting chamber are appropriate means for detecting DNA fragment denaturation, such as laser excitation at the resonant frequency of the dye label and photodiode detection. The strength and corresponding temperature of the raw absorbance or other signal is integrated by the microprocessor and the size of

analysis with a battery of precharacterized test probes. Using this method, DNA fragments are preferably prepared using *in vitro* amplification techniques, so that one strand is immobilizable due to covalent attachment of the binding molecule to one of the primers. Using this method, the DNA fragment to be tested is sequentially hybridized with and eluted by denaturation from a series of well-characterized DNA probes being detectably labeled. Alternatively (depending on the nature of the DNA mismatch expected for each probe), hybridization and denaturation are multiplexed, using probes detectably labeled with different detectable labels so that each probe can be identified. This method is useful for genetic screening as described above.

EXAMPLE 4

DNA Amplification and Analysis

Fragments of DNA are amplified *in vitro* by polymerase chain reaction (PCR) or magnetic chain reaction and analyzed by capillary electrophoresis. Reagent mixing, primer annealing, extension and denaturation in an amplification cycle resulting amplification of a 500bp target fragment and its subsequent analysis are carried out using a device and disk as described in Example 1 above. A schematic diagram of the structure of the disk is shown in Figure 21.

The disk comprises at least three sample input ports A, B and C. Port A permits injection of 30 attomoles (about 100pg) linear bacteriophage lambda DNA. Port B and C allow input of 5 μL of a 20μM solution of primer 1 and 2 respectively, having the sequence:

Primer 1: 5'-GATGAGTTCGTGTCCGTACAACTGG-3' (SEQ ID No.: 1) and

Primer 2: 5'-GGTTATCGAAATCAGCCACAGCGCC-3' (SEQ ID No.: 2).

The disk also comprises three reagent reservoirs D, E and F in the Figure and containing 54μL of distilled water; 10μL of a solution of 100mM Tris-HCl (pH 8.3), 500mM KCl, 15mM $MgCl_2$, 0.1% gelatin and 1.25μM of each dNTP; and 1μL of *Taq* DNA polymerase at a concentration of 5 Units/μL, respectively.

In addition, the disk comprises a reaction chamber G that is configured to facilitate mixing of these reagents using a flexural-plate-wave component (as described in U.S. Patent No. 5,006,749). Also included in the configuration of reaction chamber G are cooling and heating means *via* a Peltier component. These components can be integral to the disk or can be positioned in the device so as to provide heating and

capillary electrophoresis. Reagent mixing, DNA digestion and restriction fragment analysis are carried out on the disk. A schematic diagram of the structure of the disk is shown in Figure 22.

The disk comprises a sample input port A; three reagent reservoirs B, C and D; a reaction chamber E configured for mixing the reagents as described above in Example 5, and a capillary electrophoresis unit F. The reagent reservoirs contain: 1-2μL of a restriction enzyme, *e.g.* *Hin*dIII, at a concentration of 20 Units/μL in reservoir B; 4 μL of a solution of 100mM Tris-HCl (pH 7.9), 100mM $MgCl_2$ and 10mM dithiothreitol in reservoir C; and 30μL of distilled water in reservoir D. Disks are also provided that comprise a multiplicity of sets of the reaction components A through E.

Restriction enzyme digestion of the DNA is initiated by placing 4-5μL of a solution (typically, 10mM Tris-HCl, 1mM EDTA, pH 8) containing 4μg bacteriophage lambda DNA in sample input port A. The DNA sample and the reagents in reservoirs B, C and D are transferred to reaction chamber E by spinning the disk at a rotational speed of 1 to 30,000 rpm and opening valves controlling reservoirs B, C and D. The reaction is incubated at 37°C for 1h in reaction chamber E after mixing, the reaction chamber being heated by provision of a Peltier heating element either on the disk or positioned in the device so at to specifically heat the reaction chamber. After digestion, an amount of the digested DNA is transferred to electrophoresis unit F by spinning the disk at a speed of 1 to 30,000 rpm and opening a valve on reaction chamber E leading to capillary electrophoresis unit F, thereby effecting transfer of an amount of the reaction mixture to the electrophoresis unit. The amount of the reaction mixture, typically 10μL, is determined by a combination of the length of time the valve on reaction chamber E is open and the speed at which the disk is rotated. Capillary electrophoresis is accomplished as described below in Example 11, and fractionated DNA species detected using optical or other means as described above in Example 2.

EXAMPLE 6

DNA Synthesis

Oligonucleotide DNA synthesis is performed using a disk and device as described above in Example 1. Synthesis is achieved by the stepwise transport of

mixture is incubated for a defined time interval, typically 1 minute. The reaction mixture is decanted to an effluent reservoir and the CPG spun into a rinse chamber containing CH_3CN. After rinsing, the CH_3CN is decanted to an effluent reservoir and the CPG spun into a fourth reaction chamber along with a two-component "capping" reagent. The capping reaction is performed for a defined time interval, typically 1 minute. After the reaction is complete, the reaction mixture is decanted to an effluent reservoir as above and the CPG spun into a rinse chamber containing CH_3CN. The CH_3CN is then decanted to an effluent reservoir and the CPG is spun into a fifth chamber containing TCA, comprising the beginning of another cycle. The cycle is repeated by transit of the CPG through interconnected series of the four reaction chamber until the preprogrammed sequence is completely synthesized. The CPG is then spun into a reaction chamber containing concentrated ammonium hydroxide and heated at 60°C for a defined time interval, typically 6 hours, during which time the DNA molecule is deprotected and cleaved from the CPG support. The finished oligonucleotide is removed by the user or by automated means.

The disk provides a series of reaction chambers linked to each other and comprising four reaction and rinsing chambers per nucleotide to be added to the oligonucleotide chain. The disks can be loaded to produce a particular oligonucleotide, or each reaction chamber 2 can be in contact with reagent reservoirs containing each of the four nucleotide bases and linked to the reaction chamber by an individually-controllable valve. In this embodiment, activation of the appropriate valve at each step in the cycle is controlled by a signal from the device. Disks comprising a multiplicity of these synthetic arrays. Permitting simultaneous synthesis of a plurality of oligonucleotides, are also provided. A schematic diagram of a disk configured for multiple oligonucleotide synthesis is shown in Figure 23B.

DNA synthesis can also be performed upon preloaded CPG contained in reaction chambers toward the periphery of the disk and reagents delivered by the use of multiuse two-way valves, as schematically diagramed in Figure 23A. In these disks, reaction chambers capable of containing 100nL, spaced 150μm on-center (measured from the center of one sphere to the center of the next sphere) in a disk of a 120mm diameter, as many as 1250 reaction chambers can be manufactured. Reagent reservoirs containing sufficient volumes to supply the reagent chambers on the disk are prefilled with the four

port. The DNA is then transferred into a mixing chamber containing terminator solution (*i.e.*, a solution comprising a dideoxy form of nucleotides G, A, T or C) by spinning the disk at a rotational speed of 1 to 30,000 rpm. Terminator solution typically comprises 100nL of a solution containing 5 picomoles of each deoxynucleotide, 0.5 picomoles of one dideoxynucleotide covalently linked to a fluorescent label, 90mM Tris-HC1-(pH 7.5), 45mM $MgCl_2$ and 110mM NaCl. The contents of the mixing chamber are transferred into a reaction chamber containing 0.1 units of T7 DNA polymerase (or, alternatively, 0.1 Units of *Taq* polymerase) and 20nL 0.1M dithiothreitol (DTT) by spinning the disk at a rotational speed of 1 to 30,000 rpm, yielding a reaction mixture in the reaction chamber having a final concentration of buffer components that is 26mM Tris- HC1 (pH 7.5), 13mM MgCl 2, 32mM NaCl, and 6mM DTT. The reaction chamber is heated to 37°C (or, alternatively, to 65°C for Taq polymerase) by a resistive heating element integral to the disk, or alternatively, positioned within the device to specifically heat the reaction chamber, and incubated for a defined length of time, typically 1 minute. The reaction products are spun into an equal volume of 90% formamide/EDTA, heated to 90°C for 1 minute and spun to a capillary electrophoresis unit on the disk. The set of dideoxynucleotide-terminated DNA fragments comprising the reaction mixture is then separated by capillary electrophoresis and the sequence of fragments determined by laser-induced fluorescence detection as described above. Disks comprising a multiplicity of these synthetic arrays, permitting simultaneous synthesis of a plurality of dideoxynucleotide-terminated oligonucleotides, are also provided. The deducted nucleotide sequence is determined from the pattern of fluorescence signals detected and the sequence is determined from the pattern of fluorescence signals detected and the sequence derived by the device microprocessor from these data.

EXAMPLE 8

Liquid phase synthesis and analysis

A variety of colorimetric chemical analyses are performed using a disk as described in Example 1. For example, a disk is provided (*see* Figure 25) for performing a solution assay to determine iron concentration in a test solution (such as an industrial effluent) using a standard colorimetric test. The device is fabricated with reagent reservoir containing 40uL 12N HC1, 100uL 10% hydroxylamine hydrochloride, 100uL

in a funnel at the end of the reaction chamber and deposited to waste. A valve controlling an imidazole reagent chamber containing 50 uL of 0.1M imidazole is then opened above the particles but below the decanting level and used to transfer the particles through a valve in the reaction chamber and into the next decanting reservoir. This decanting process can be repeated many times to affect a change in the liquid phase to the desired composition. Typically, three exchanges are sufficient. Alternatively, appropriate configuration of the reagent and reaction chambers allows the magnetic particles to be exchanged within a single reaction chamber by controlled addition and removal of imidazole from clusters of reagent reservoirs, or alternatively, a single reagent reservoir large enough to contain sufficient imidazole for the entire cycle of exchange.

After the exchange cycle is complete, the magnetic particles are transferred to a next reaction chamber containing 250 ug dry 1-ethyl-3(3-dimenthylaminopropyl) carbodiimide (EDAC). A reagent reservoir containing 170 OD (170ng) 5'-aminated DNA oligonucleotide in 50 uL of 0.1 M imidazole solution chamber prior to addition of the particles in order to dissolve the EDAC. The particles are then added through a valve in about 100 uL 0.1 M imidazole. Upon addition of the magnetic particles to the reaction chamber, the device is stopped and incubated 6 hours at 40°C. Heating can be effected by a heat source (such as Peltier heating device) embedded in the disk itself, or positioned in the instrument in a configuration permitting specific heating of the reaction chamber. In the latter alternative, the disk may be stopped at a predetermined position relative to the device to ensure specificity of heating of the reaction chamber.

After incubation, the particles are washed and exchanged into 100 uL portions of water by decanting as described above as the disk is spun. Three exchanges are typically performed to purify the particles. Product is advantageously collected in the extremity of the disk where it can easily be accessed for subsequent use. Disks comprising a multiplicity of these synthetic arrays, permitting simultaneous synthesis of a plurality of particle-linked oligonucleotides, are also provided.

EXAMPLE 10

Micro-Extraction System

A disk as described in Example 1 (see Figure 27) for performing micro-

drop is applied between the sample inlet and outlet ports while the separation channel ports float. The sample, comprising a solution of 5mM EDTA, 1mM Tris-HCl (pH 8) with 1 mM Mg^{2+} and 1 mM Ca^{2+} (typically prepared from the chloride salt). The running buffer consists of 10 mM Tris-HCl (pH 8), 5 mM EDTA. Separation toward the cathode is then performed by floating the electric potential at the sample reservoir and applying 250 V along the separation channel. Separation is monitored at a position 2 cm from the inlet port by monitoring, e.g. UV absorbance at 254 nm using a UV light source (mercury lamp) and a photodiode detector, positioned on the device to interest the capillary channel.

EXAMPLE 12

DNA electrophoresis

Gel electrophoresis is performed on a disk prepared as described in Example 1 above. For this application, a gel media is prepared in the separation channel; however, such gel media must be protected from sheer forces that develop with rotation of the disk during transfer of sample or buffer to the electrophoresis channel. Thus, the gel-filled capillary is advantageously arrayed concentrically on the disk, as shown schematically in Figure 29. As a result, the gel will only experience shear forces from centripetal-induced pressure during rotation if a fluid reservoir is in contact with the capillary during rotation of the disk. At rest, the planar geometry of the disk prevents hydrodynamic pressure on the capillary. This is an advantage over standard capillary electrophoresis systems, where hydrodynamic pressure is not so easily controlled because the buffer volumes are reservoir heights need to be carefully adjusted before each run to avoid hydrodynamic flow. This is also an advantage of capillary electrophoresis performed on the disks of the invention over electrophoresis performed on microchips, where buffer reservoirs are positioned above the plane of the separation channel and are thereby susceptible to hydrodynamic pressure-driven fluid flow.

Gel electrophoresis is performed on the disks of the invention to separate DNA fragments, including duplex PCR fragments, oligonucleotides and single-stranded , dideoxynucleotide-terminated enzymatic DNA sequencing components, the system is configured as shown in Figure 29. The disk is prepared comprising a polyacrylamide gel concentrically arrayed in a microetched separation channel in the disk. The

is dependent on the depth of the absorbing layer, as well as the concentration of the absorbing molecules (as described in the Lambert-Beer law).

Although a measurement cell in a rotating microsystem platform of the invention presents a short transverse pathlength, the lateral pathlength through the disk can be extensive (*i.e.*, centimeters *versus* millimeters). Spectral measurements can be enhanced by introducing light through the detection chamber in the lateral dimension.

One arrangement providing transverse illumination in the lateral dimension is shown in Figure 16. Light is beamed in a perpendicular direction towards the disk. A mirror is positioned at a 45° angle to the direction of the illuminating beam, whereby the light is directed laterally through the detection chamber. Light passes through the detection cell and is redirected by another 45° mirror onto a photosensitive detector, such as a photodiode or photomultiplier tube. These mirrors can be inserted onto the disk, integrally molded into the disk or metallicized in the plastic or other substrate comprising the disk.

EXAMPLE 14

Cell Counting, Identification and Monitoring

Methods for identifying particular cells or cell types in a biological sample are provided. For example, a microplatform of the invention is prepared by having a surface adsorbly coated with monoclonal antibody specific to *E. coli.*, the remaining sites being blocked with BSA. A milk sample is introduced onto the disk and placed into contact with a reaction chamber comprising the surface coated with the antibody. The milk is incubated in this chamber for 30 min. The microsystem platform is then rotated to remove unwanted materials. An amount of a buffer appropriate for washing the microsystem chamber is then added to the surface or chamber through a microchannel from a reservoir containing washing buffer, said buffer being released by centrifugal force and opening of a microvalve. In a useful embodiment, the washing buffer comprises an *E. coli*-specific monoclonal antibody crosslinked to an enzyme (such peroxidase). Thus incubation is allowed to proceed for 5 min. The disk is again spun with the opening of th appropriate microvalves to remove the washing solution from the chamber and to add a solution containing an enzymatic substrate (tetramethylbenzidine

What is claimed is:

1. A centripetally-motivated fluid micromanipulation apparatus that is a combination of

a microsystem platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, wherein the first surface comprises a multiplicity of microchannels embedded therein and a sample input means, wherein the sample input means and the microchannels are connected and in fluidic contact, and wherein the second flat, planar surface opposite to the first flat planar surface of the platform is encoded with an eletromagnetically-readable instruction set for controlling rotational speed, duration, or direction of the platform, and

a micromanipulation device, comprising a base, a rotating means, a power supply and user interface and operations controlling means, wherein the rotating means is operatively linked to the microsystem platform and in rotational contact therewith

wherein a volume of a fluid within the microchannels of the platform is moved through said microchannels by centripetal force arising from rotational motion of the platform for a time and a rotational velocity sufficient to move the fluid through the microchannels.

2. A centripetally-motivated fluid micromanipulation apparatus that is a combination of

a microsystem platform, comprising a substrate having a first flat, planar surface and a second flat, planar surface opposite thereto, wherein the first surface comprises a multiplicity of microchannels, a reaction chamber and a reagent reservoir embedded therein, and a sample input means, wherein the sample input means, the microchannels, the reaction chamber and the reagent reservoir are connected and in fluidic contact, and wherein the second flat, planar surface opposite to the first flat planar surface of the platform is encoded with an eletromagnetically-readable instruction set for controlling rotational speed, duration, or direction of the platform, and

a micromanipulation device, comprising a base, a rotating means, a power supply and user interface and operations controlling means, wherein the rotating means is operatively linked to the microsystem platform and in rotational contact therewith

wherein a volume of a fluid within the microchannels of the platform is moved

6. The apparatus of Claim 1, wherein the microsystem platform is constructed of an material selected from the group consisting of an organic material, an inorganic material, a crystalline material and an amorphous material.

7. The apparatus of Claim 6, wherein the microsystem platform is further comprises a material selected from the group consisting of silicon, silica, quartz, a ceramic, a metal or a plastic.

8. The apparatus of Claim 4, wherein the microsystem platform is a disk having a radius of about 1 to 25cm.

9. The apparatus of Claim 1, wherein the microsystem platform has a thickness of about 0.1 to 100mm, and wherein the cross-sectional dimension of the the microchannels between the first and second flat, planar surfaces is less than 500μm and from 1 to 90 percent of said cross-sectional dimension of the platform.

10. The apparatus of Claim 10, wherein the microsystem platform has a thickness of about 0.1 to 100mm, and wherein the cross-sectional dimension of the reaction chamber or the reagent reservoir between the first and second flat, planar surfaces is from 1 to 75 percent of said thickness of the platform.

11. The apparatus of Claim 1, wherein the microsystem platform is rotated at a rotational velocity of about 1 to about 30,000rpm.

12. The apparatus of Claim 1, wherein the microsystem platform comprises a multiplicity of sample input means, reagent reservoirs, reaction chambers and microchannels connected thereto and embedded therein, wherein a volume of a fluid containing a sample is moved on the disk from the sample input means into and out from the reaction chambers, and a volume of a reagent is moved from the reagent reservoirs into and out from the reaction chambers, by centripetal force arising from rotation of the microsystem platform.

22. The apparatus of Claim 1, further comprising a temperature controlling element in thermal contact with the microplatform.

23. The apparatus of Claim 1 further comprising a thermal detecting unit in thermal contact with the microplatform.

24. The apparatus of Claim 1, wherein the microsystem platform comprises a filtering means linked to a microchannel.

25. The apparatus of Claim 1, wherein the microsystem platform comprises a mixing element connected to a reaction reservoir or a microchannel.

26. The apparatus of Claim 25, wherein the microsystem platform comprises a static mixer comprising a textured surface of a reaction reservoir or microchannel.

27. The apparatus of Claim 3, wherein the microsystem platform comprises a multiplicity of microvalves operatively linked to the microchannels, reaction reservoirs, reagent chambers, sample input means and sample outflow ports, wherein fluid flow on the microsystem platform is controlled by opening and closing the microvalves.

28. The apparatus of Claim 27, wherein the microsystem platform comprises a capillary microvalve connected to a reaction chamber or microchannel.

29. The apparatus of Claim 1, wherein the microsystem platform comprises a multiplicity of air channels, exhaust air ports and air displacement channels.

30. The apparatus of Claim 1, wherein the rotating means of the device is an electric motor.

31. The apparatus of Claim 1, wherein the device comprises a rotational motion controlling means for controlling the rotational acceleration and velocity of the

41. The apparatus of Claim 1, wherein the micromanipulation device includes a read-only memory or permanent storage memory that is encoded with machine language instructions.

42. The apparatus of Claim 41, wherein the machine language instructions control operation of the platform, data acquisition or analysis from the platform, data storage and retrieval, communication to other devices, or direct apparatus performance diagnostics.

43. The apparatus of Claim 1 further comprising first and second microsystem platforms in contact with one another across one planar surface of each microsystem platform.

44. The apparatus of Claim 1, wherein the microsystem platform is rotated at a velocity of from about 1 to about 30,000rpm.

45. The apparatus of Claim 1, wherein fluid on the microsystem platform is moved within the microchannels of the platform with a fluid velocity of from about 0.1cm/sec to about 1000cm/sec.

46. An apparatus according to Claim 1 for measuring the amount of an analyte in a biological sample, wherein the microsystem platform comprises

a multiplicity of sample inlet ports, arranged concentrically around the center of the platform, wherein each of the sample inlet ports is operatively linked to

a multiplicity of microchannels arrayed radially away from the center of the platform, said microchannels being operatively linked to

a multiplicity of reagent reservoirs containing a reagent specific for the analyte to be measured, wherein release of the reagent from each of the reservoirs is controlled by a microvalve, and wherein the multiplicity of microchannels is also operatively linked to

a multiplicity of analyte detection chambers arranged peripherally around the outer edge of the microplatform,

monochromatic light source.

56. The apparatus of Claim 46 , wherein the detecting means detects fluorescence, chemiluminescence, light-scattering or radioactivity.

57. A method for measuring the amount of an analyte in a biological sample, the method comprising the steps of

applying the biological sample to a sample inlet port of the microsystems platform of Claim 46,

placing the microsystems platform in a micromanipulation device,

providing rotational motion to the microsystems platform for a time and at a velocity sufficient to motivate the biological sample containing the analyte from the sample inlet port through the microchannel,

opening each of the microvalves controlling release of the reagent from the reagent reservoirs by generating a signal from the controlling unit, at a time and for a duration whereby the reagent moves into the microchannel and is mixed with the biological sample,

observing the mixture of the biological sample and the reagent in the analyte detection chamber, whereby a detector comprising the device detects a signal proportional to the amount of the analyte present in the biological sample, and

recording the measurement of the amount of the analyte in the biological sample.

58. The method of Claim 57, wherein the biological sample is blood, urine, cerebrospinal fluid, plasma, saliva, semen, or amniotic fluid.

59. The method of Claim 57, wherein the measurment of the amount of analyte in the sample is recorded in the device, on the microplatform, or both.

60. The method of Claim 57, wherein the analyte detection chamber on the microsystem platform is optically transparent.

61. The method of Claim 57, wherein the signal detected is the analyte

chip.

67. The apparatus of Claim 64, wherein the detector comprises an optically-transparent particle collection chamber.

68. The apparatus of Claim 67, wherein the detector also comprises a coherent light source.

69. The apparatus of Claim 68, wherein the particles are detected by light scattering.

70. The apparatus of Claim 64, wherein the detector comprises a particle collection chamber operatively connected by a microchannel to a reagent reservoir comprising a reagent for chemically testing the particles.

71. A method for detecting gas or particles comprising an environmental sample, wherein the method comprises the steps of

contacting the environmental sample with a sample inlet port of the microsystems platform of Claim 64,

placing the microsystems platform in a micromanipulation device,

providing rotational motion to the microsystems platform for a time and at a velocity sufficient to motivate the gaseous or particulate environmental sample from the sample inlet port through the microchannel,

opening each of the microvalves controlling release of the reagent from the reagent reservoirs by generating a signal from the controlling unit, at a time and for a duration whereby the reagent moves into the microchannel and is mixed with the environmental sample,

detecting the mixture of the environmental sample and the reagent or the gaseous or particulate component of the environmental sample directly in the gas or particle detection chamber, whereby the detector detects a signal proportional to the amount of the gas or particulate present in the environmental sample, and

recording the measurement of the amount of the gas or particulate in the

platform.

79. An apparatus of Claim 78, wherein the coherent light source is mounted on a movable track arrayed radially from the center of rotation of the platform.

80. An apparatus of Claim 78 further comprising a Clarke electrode operatively connected to each of the microchannels of the microsystem platform, wherein the electrode is in contact with a blood sample within the microchannel.

81. An apparatus of Claim 78 further comprising a Severing electrode operatively connected to each of the microchannels of the microsystem platform, wherein the electrode is in contact with a blood sample within the microchannel.

82. A method for determining a hematocrit value from a blood sample, the method comprising the steps of

applying the blood sample to the proximal end of a microchannel of the microsystems platform of Claim 78,

placing the microsystems platform in a micromanipulation device,

providing rotational motion to the microsystems platform for a time and at a velocity sufficient to motivate the red blood cells comprising the blood sample to move along the extent of the microchannel,

scanning the microchannel along its length with the coherent light source,

detecting a change in light scatter at a position along the microchannel that defines a boundary between the red blood cells and blood plasma,

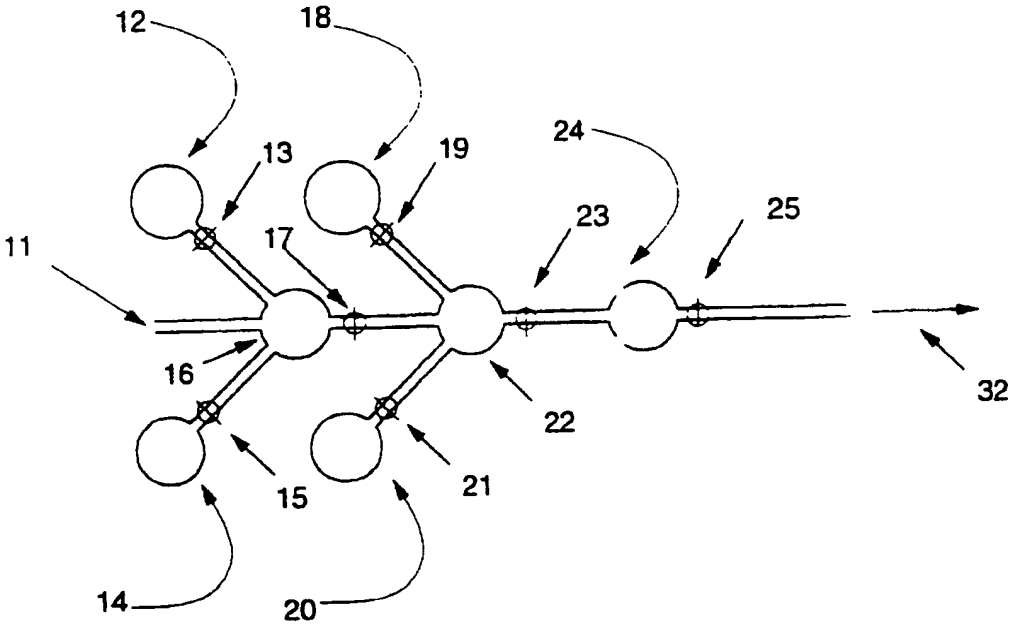
recording the position of the boundary for each microchannel, and

comparing the position of this boundary for each microchannel with a standard curve relating hematocrit values to the position of the boundary, and recording the hematocrit determined thereby.

83. A method for determining a blood oxygenation value from a blood sample, the method comprising the steps of

applying the blood sample to the proximal end of a microchannel of the

FIG. 1A



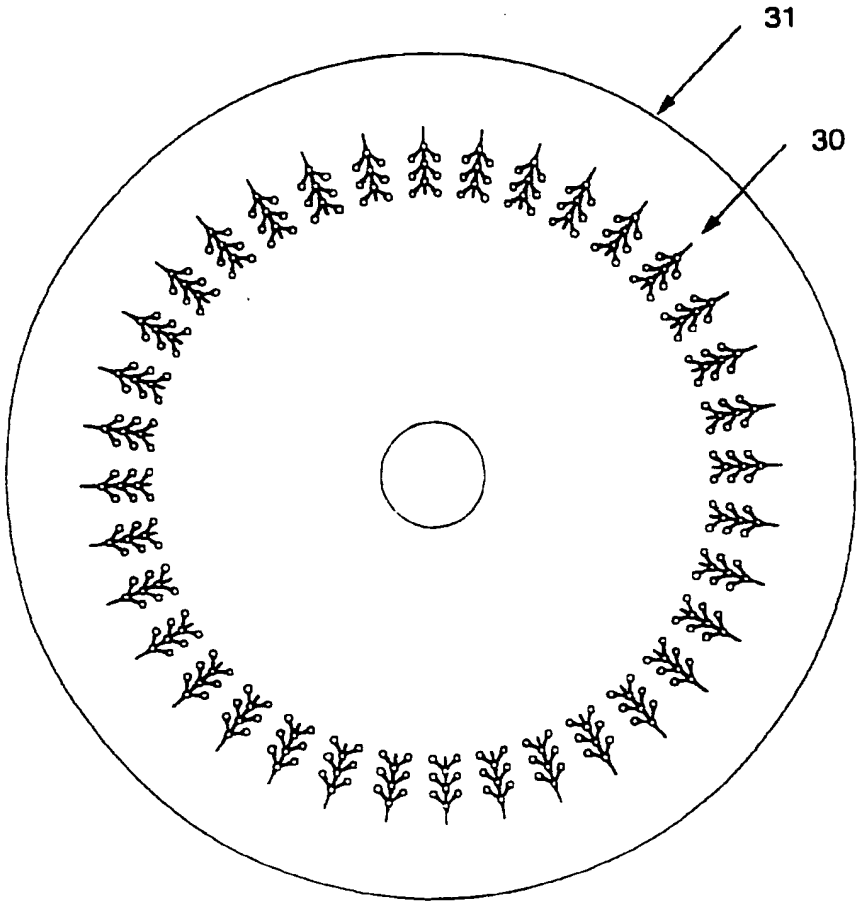


FIG. 1C

FIG. 2A

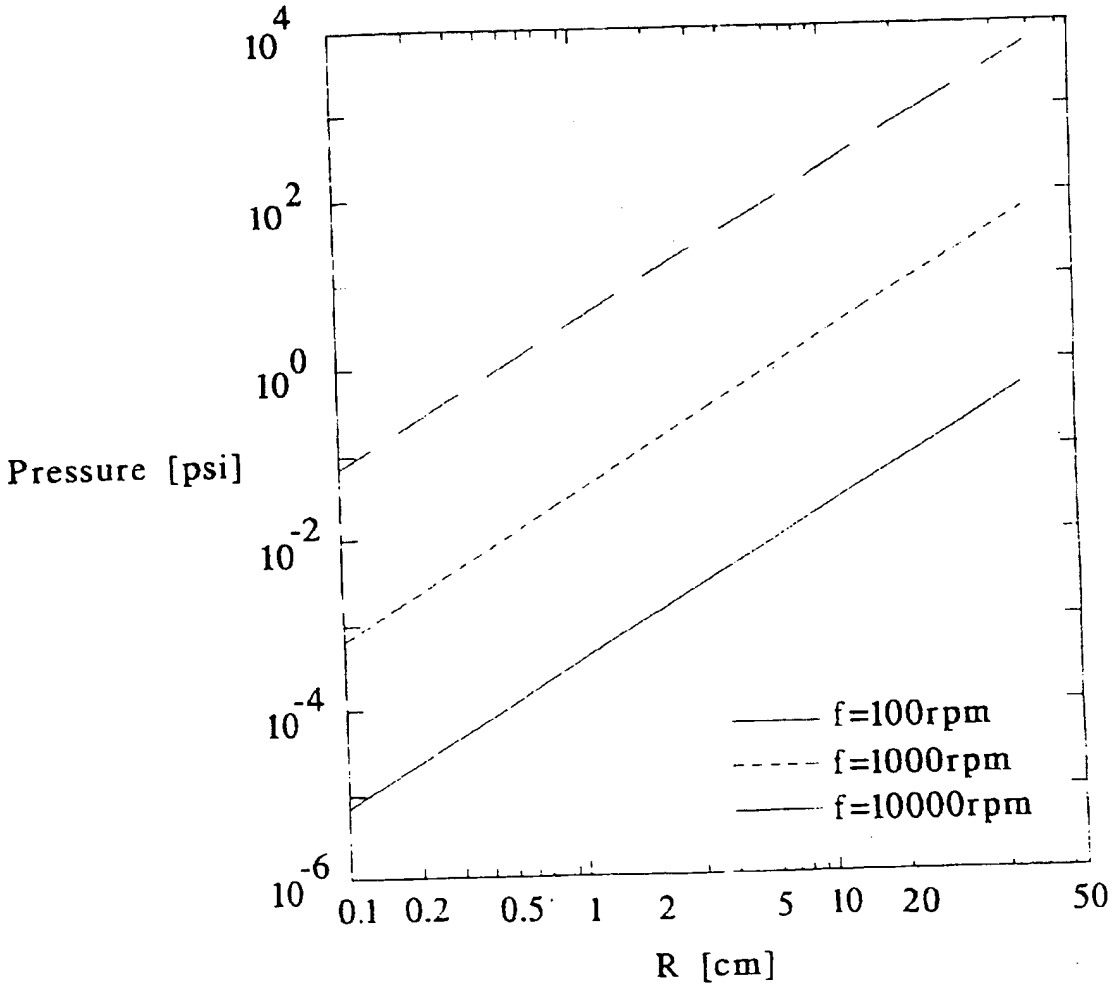

Pressure [psi]
10^4
10^2
10^0
10^-2
10^-4
10^-6
0.1
0.2
0.5
1
2
5
10
20
50
R [cm]
f=100rpm
f=1000rpm
f=10000rpm

FIG. 3A

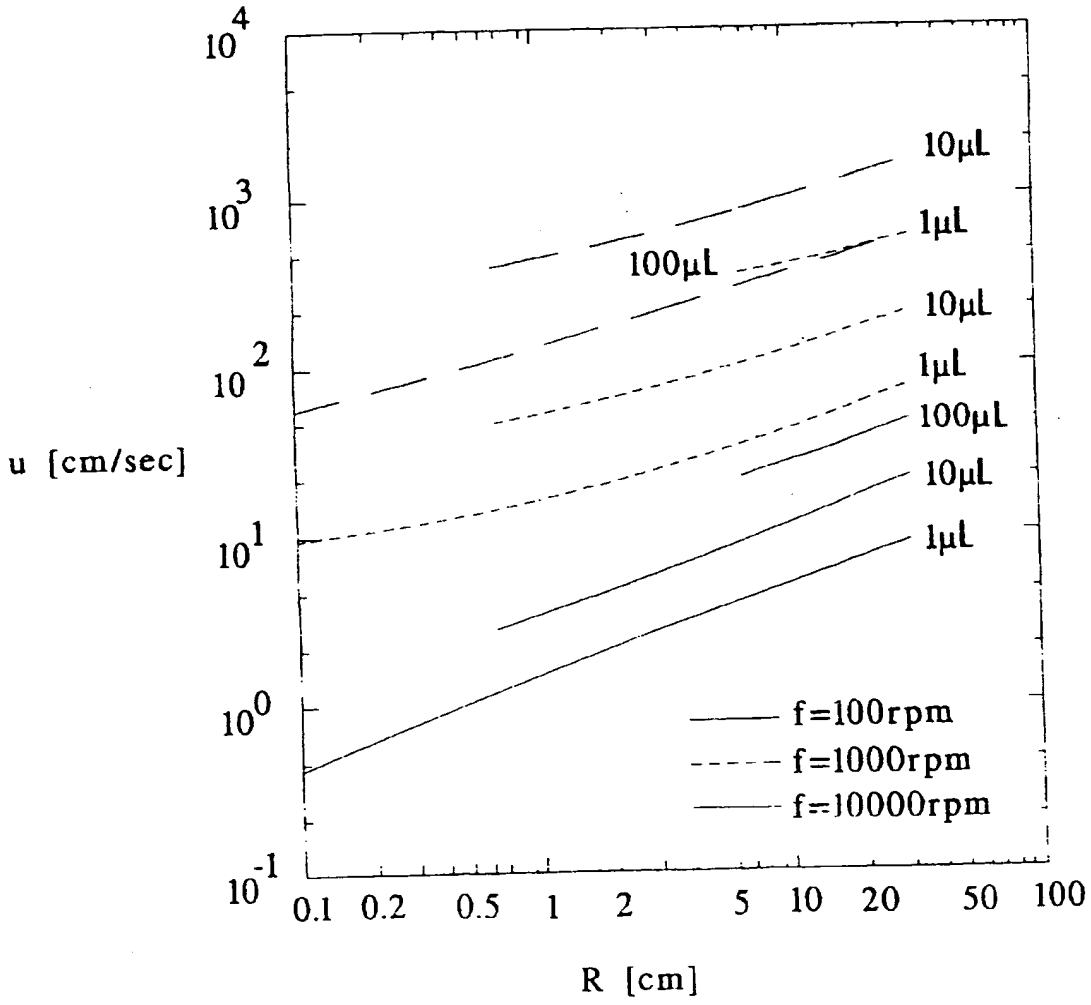

10^4
10^3
10^2
10^1
10^0
10^{-1}
u [cm/sec]
10μL
1μL
100μL
10μL
1μL
100μL
10μL
1μL
f=100rpm
f=1000rpm
f=10000rpm
0.1 0.2 0.5 1 2 5 10 20 50 100
R [cm]

FIG. 4A

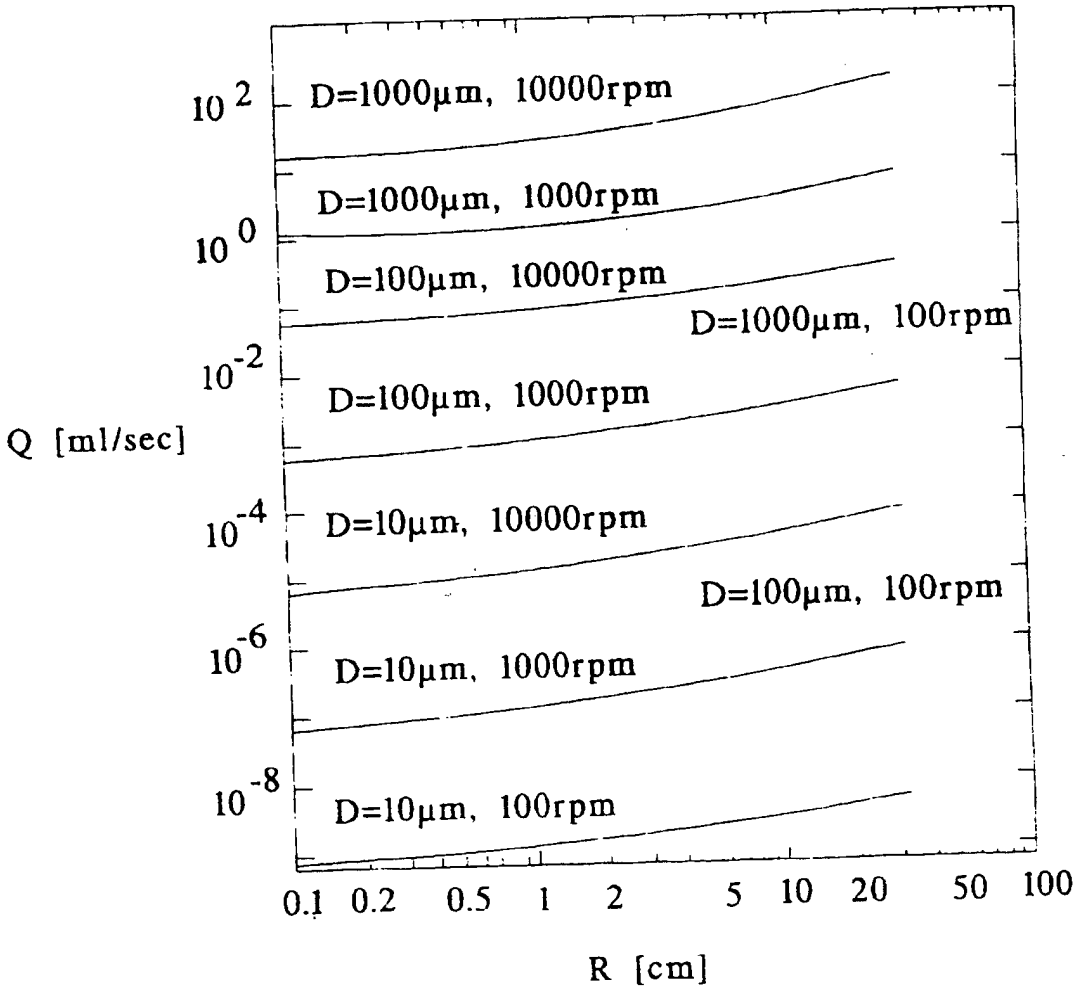

D=1000µm, 10000rpm
D=1000µm, 1000rpm
D=100µm, 10000rpm
D=1000µm, 100rpm
D=100µm, 1000rpm
D=10µm, 10000rpm
D=100µm, 100rpm
D=10µm, 1000rpm
D=10µm, 100rpm
10 2
10 0
10-2
10-4
10-6
10-8
Q [ml/sec]
0.1 0.2 0.5 1 2 5 10 20 50 100
R [cm]

FIG. 5A

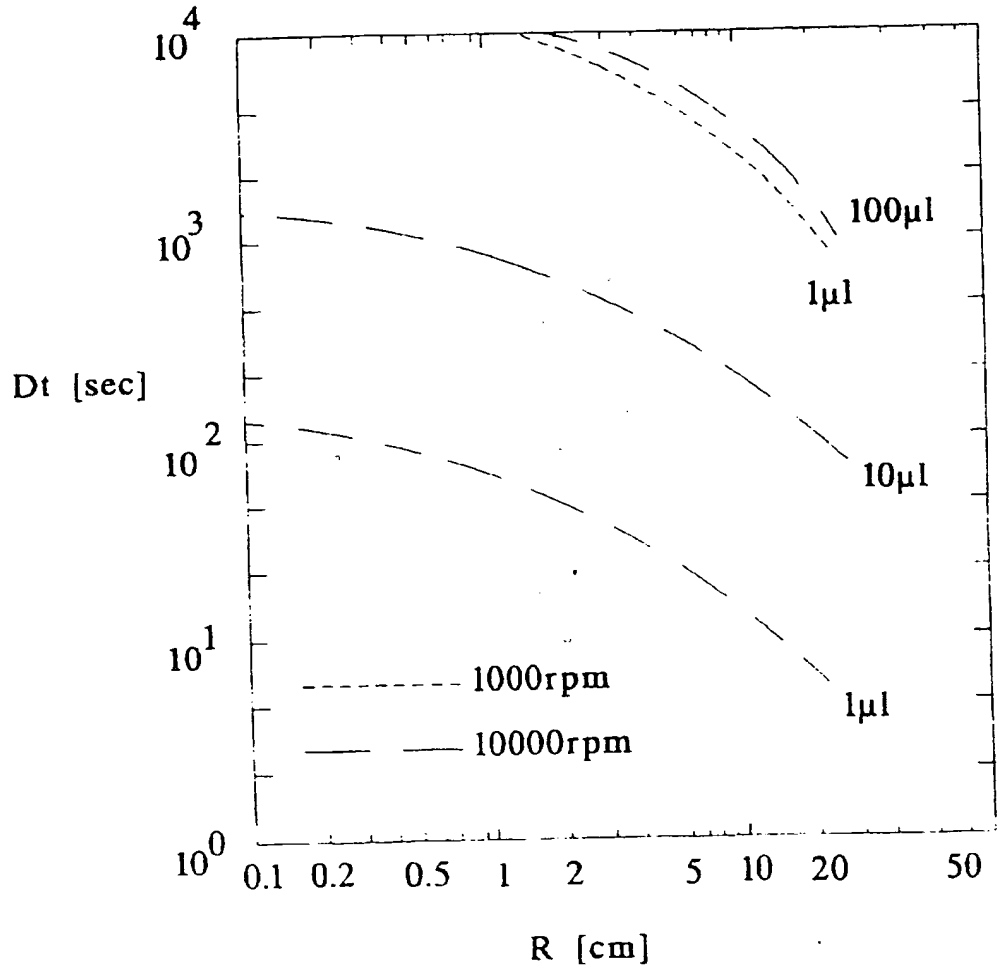

10^4
10^3
10^2
10^1
10^0
Dt [sec]
100µl
1µl
10µl
1µl
1000rpm
10000rpm
0.1 0.2 0.5 1 2 5 10 20 50
R [cm]

FIG. 5C

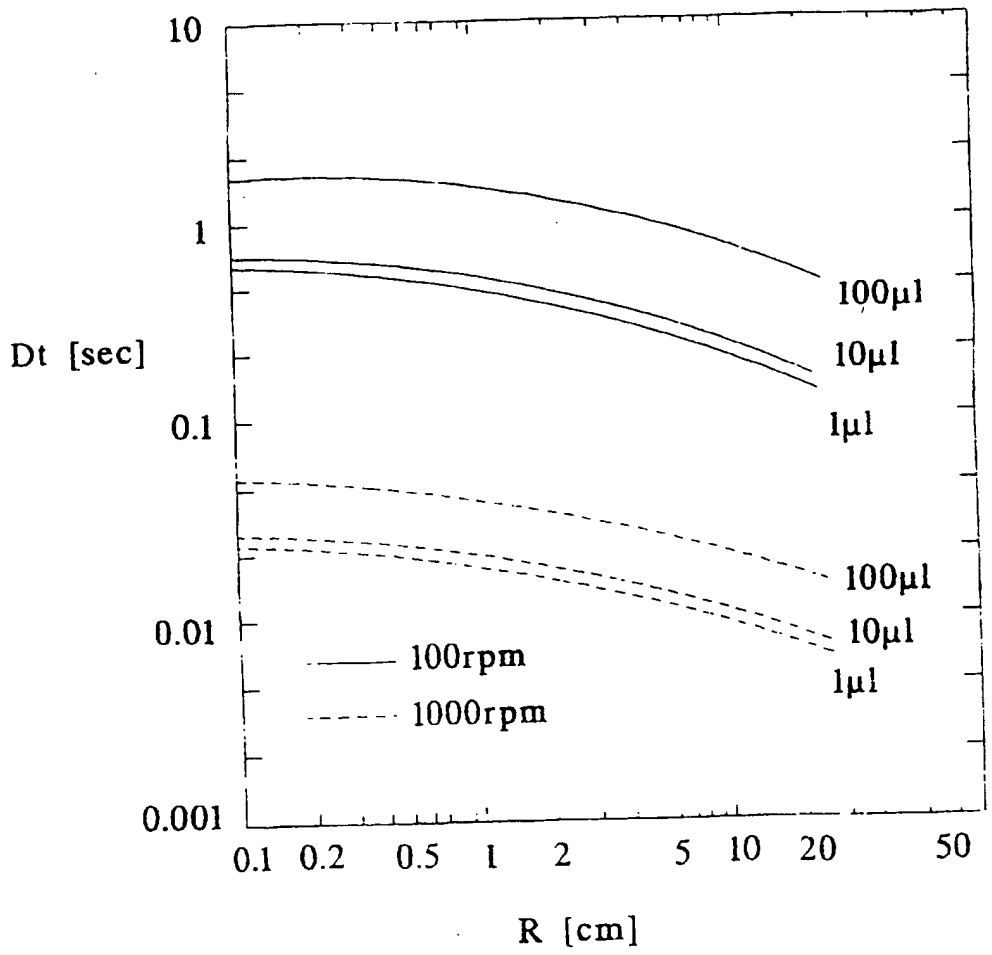
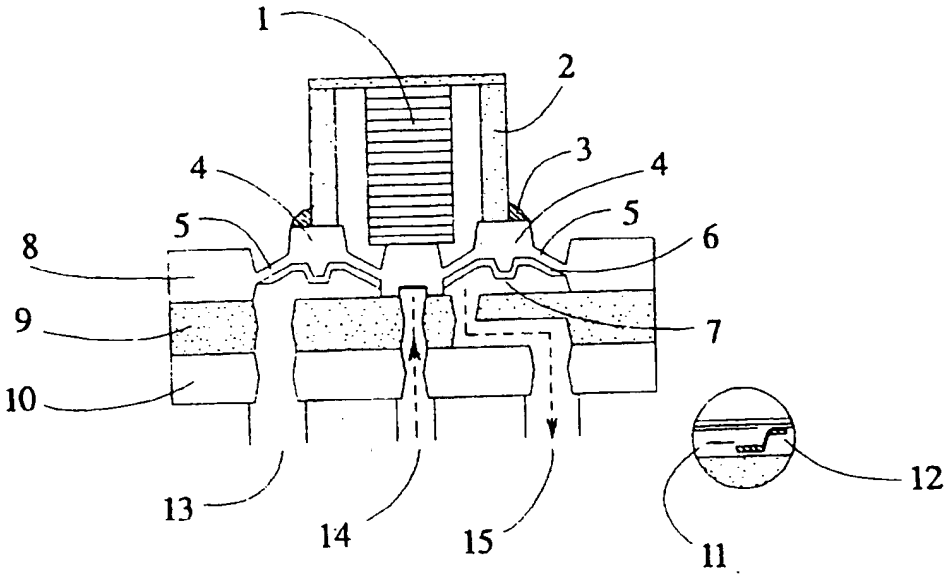

10
1
0.1
0.01
0.001
Dt [sec]
100µl
10µl
1µl
100µl
10µl
1µl
100rpm
1000rpm
0.1 0.2 0.5 1 2 5 10 20 50
R [cm]

FIG. 6



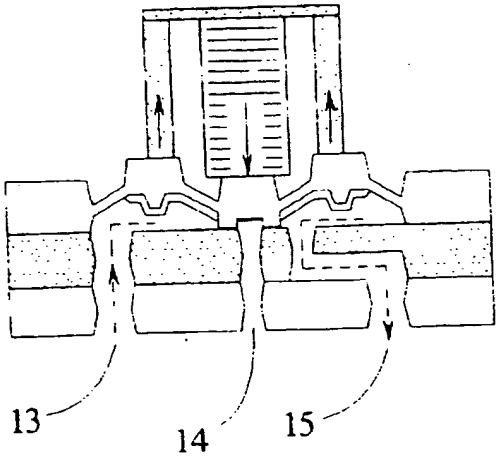


FIG. 8

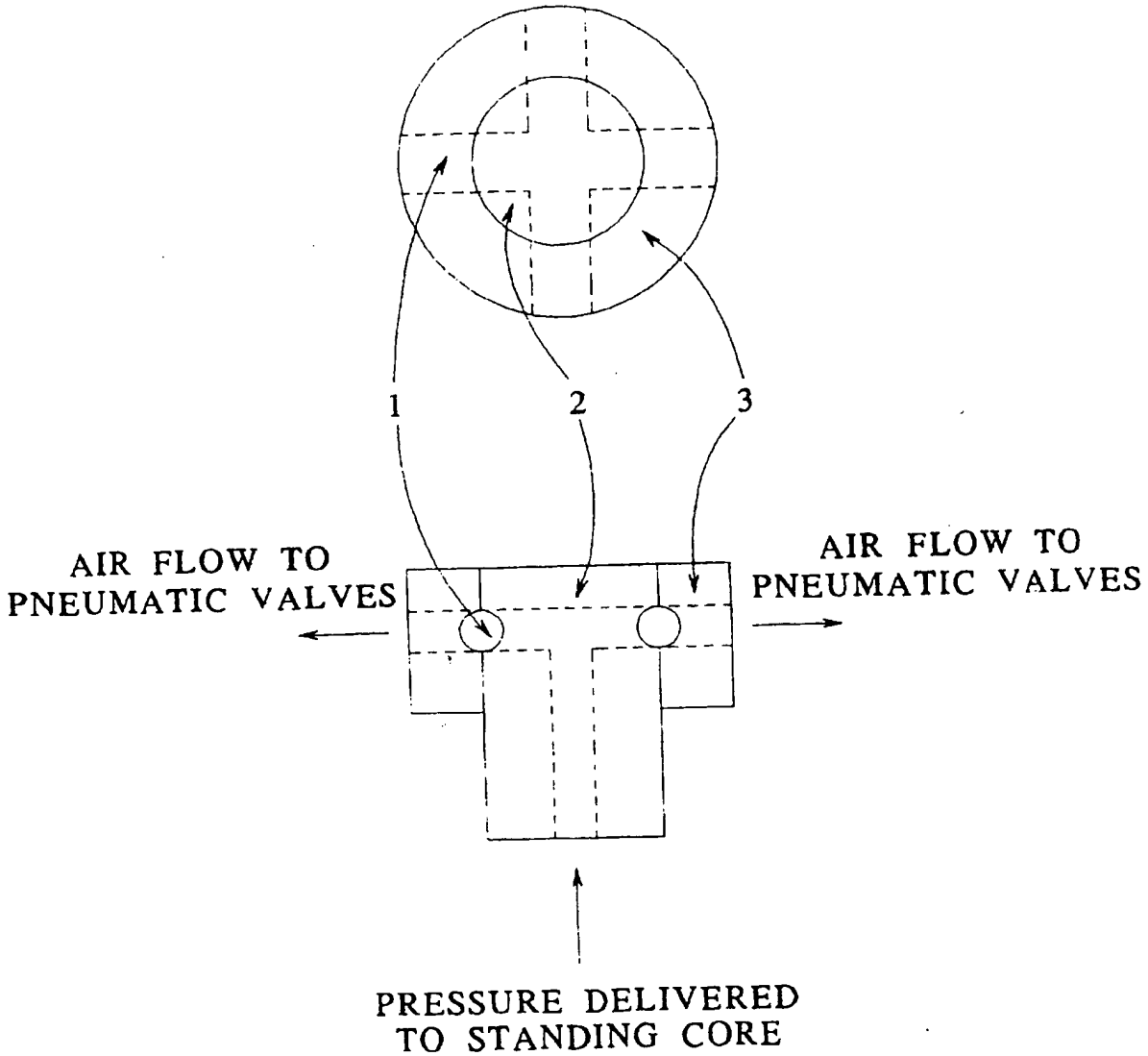

1
2
3
AIR FLOW TO PNEUMATIC VALVES
AIR FLOW TO PNEUMATIC VALVES
PRESSURE DELIVERED TO STANDING CORE

FIG. 10

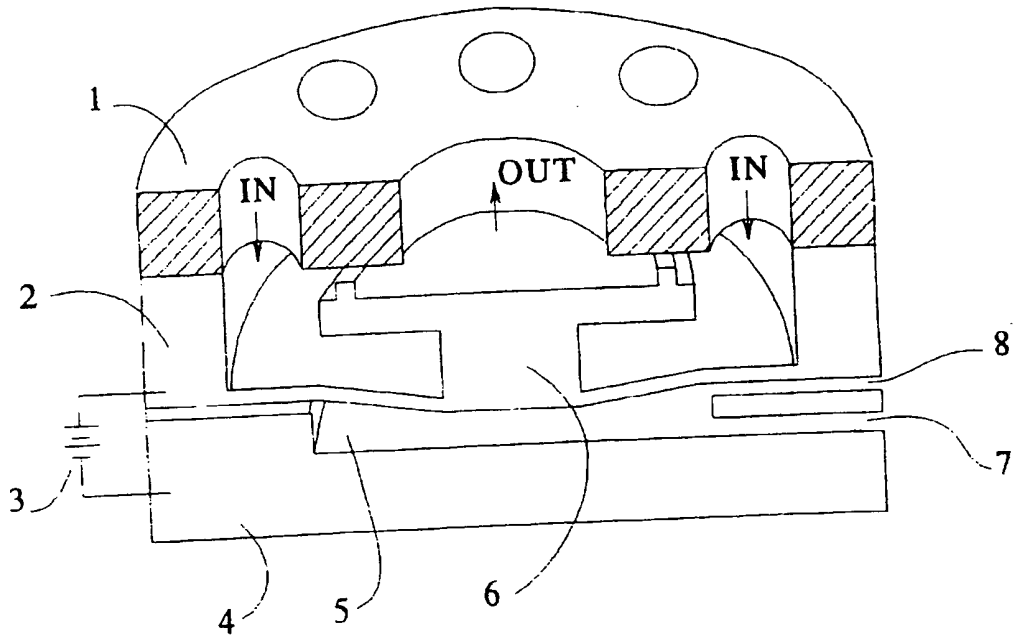


FIG. 12A

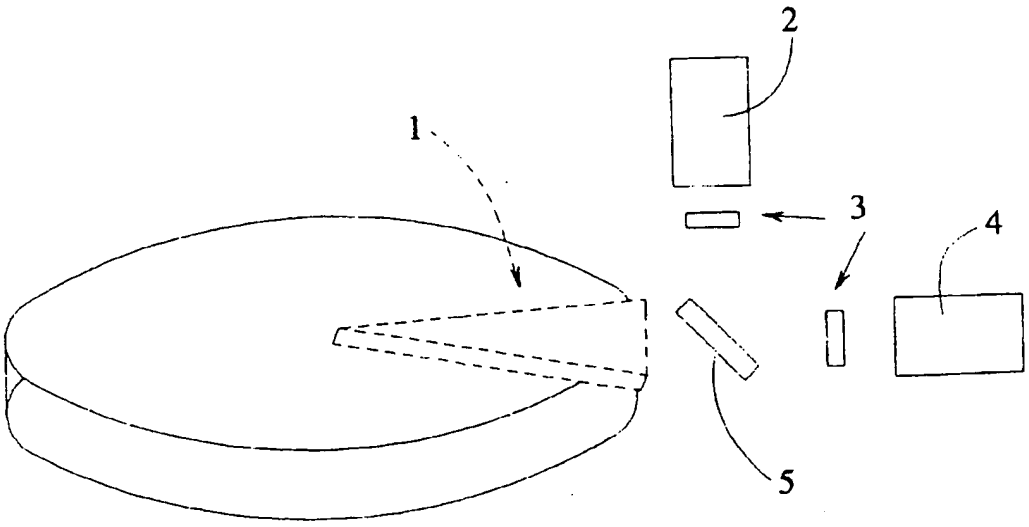


FIG. 12B

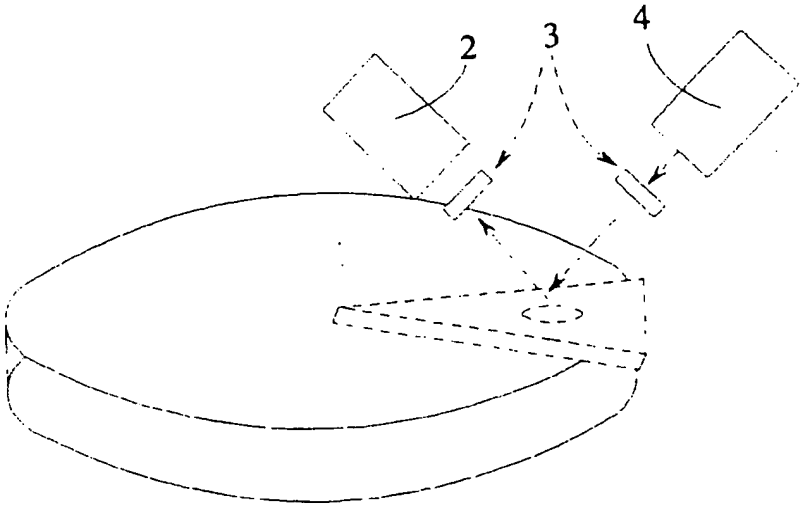
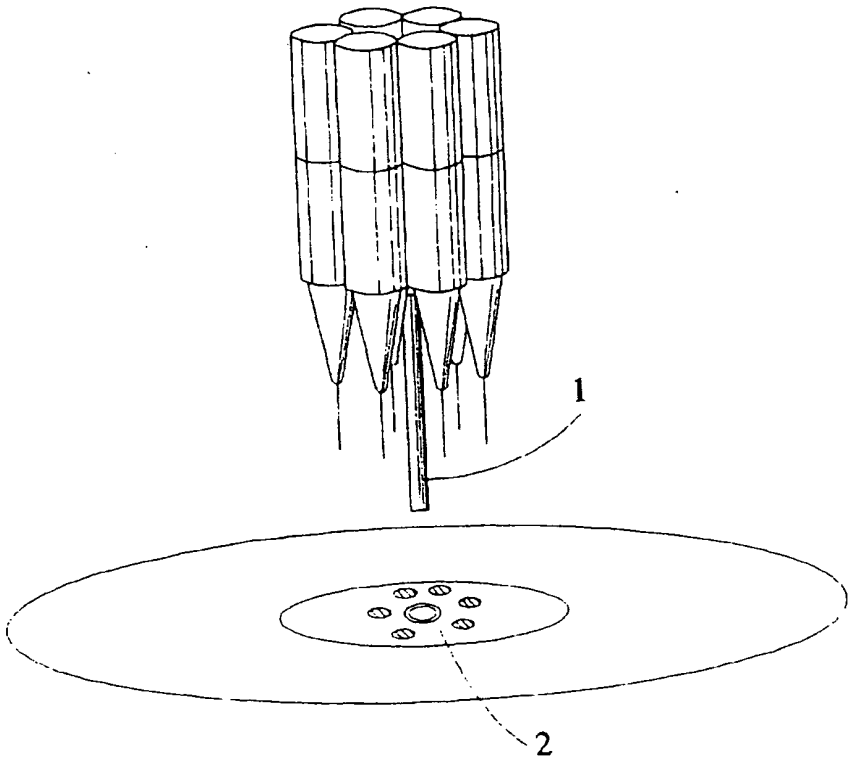


FIG. 13C

RADIAL
CONFIGURATION



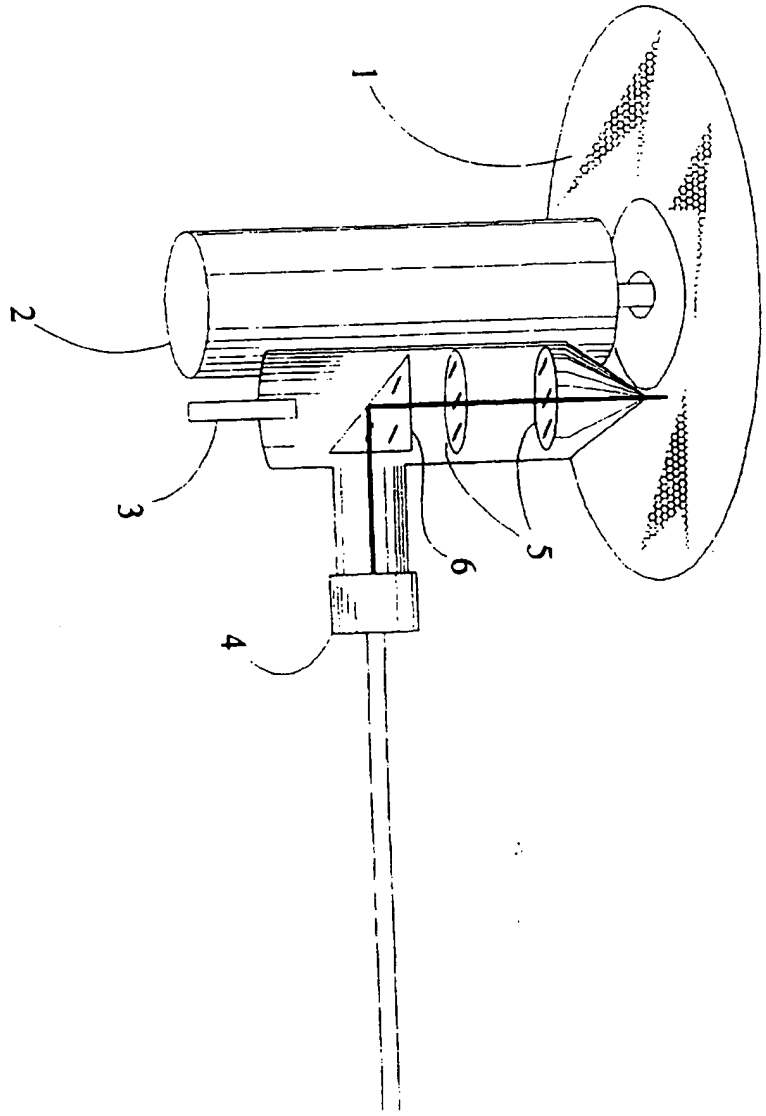


FIG. 14B

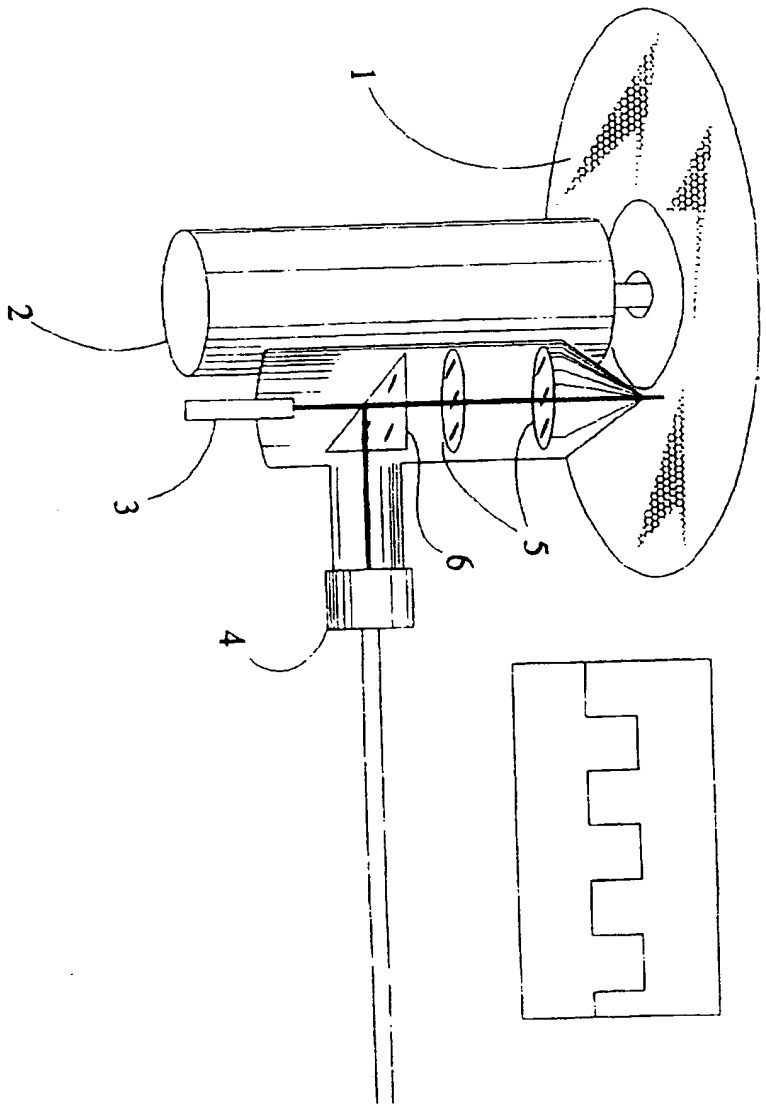


FIG. 14D

The photo diode converts the pulses into an electrical signal.

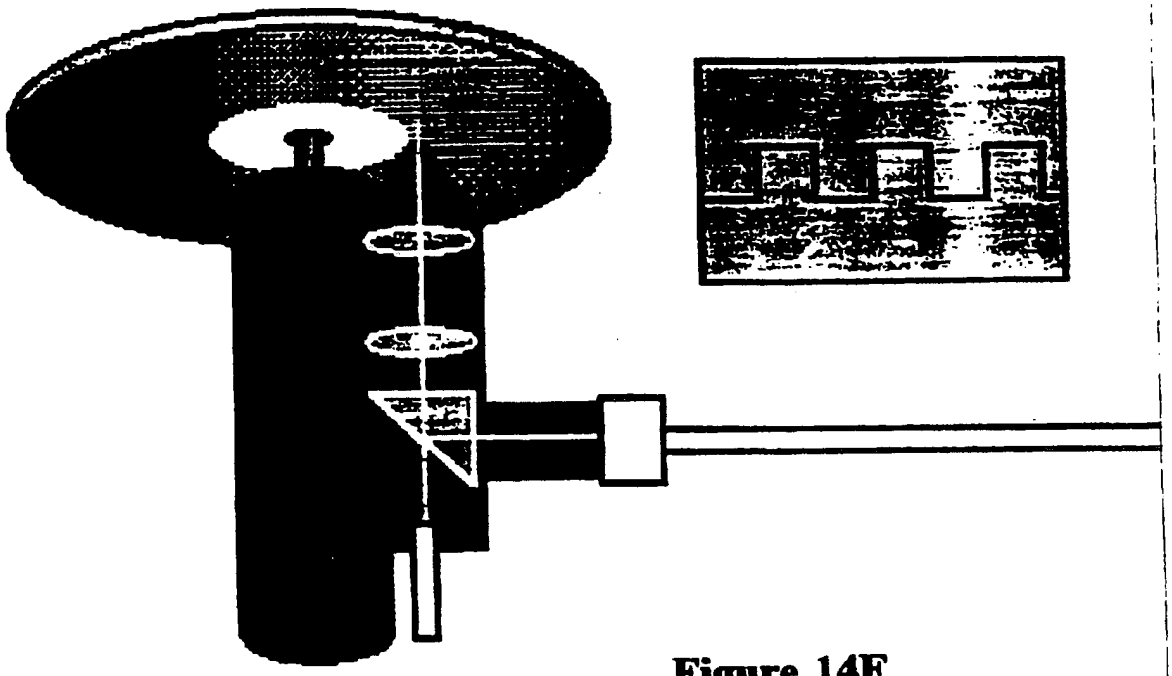


Figure 14F

FIG. 16

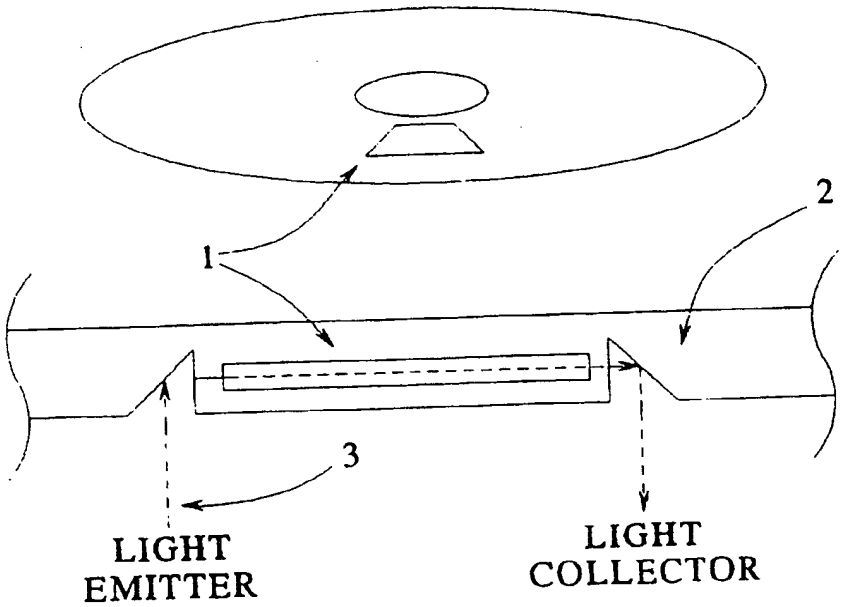


FIG. 17B

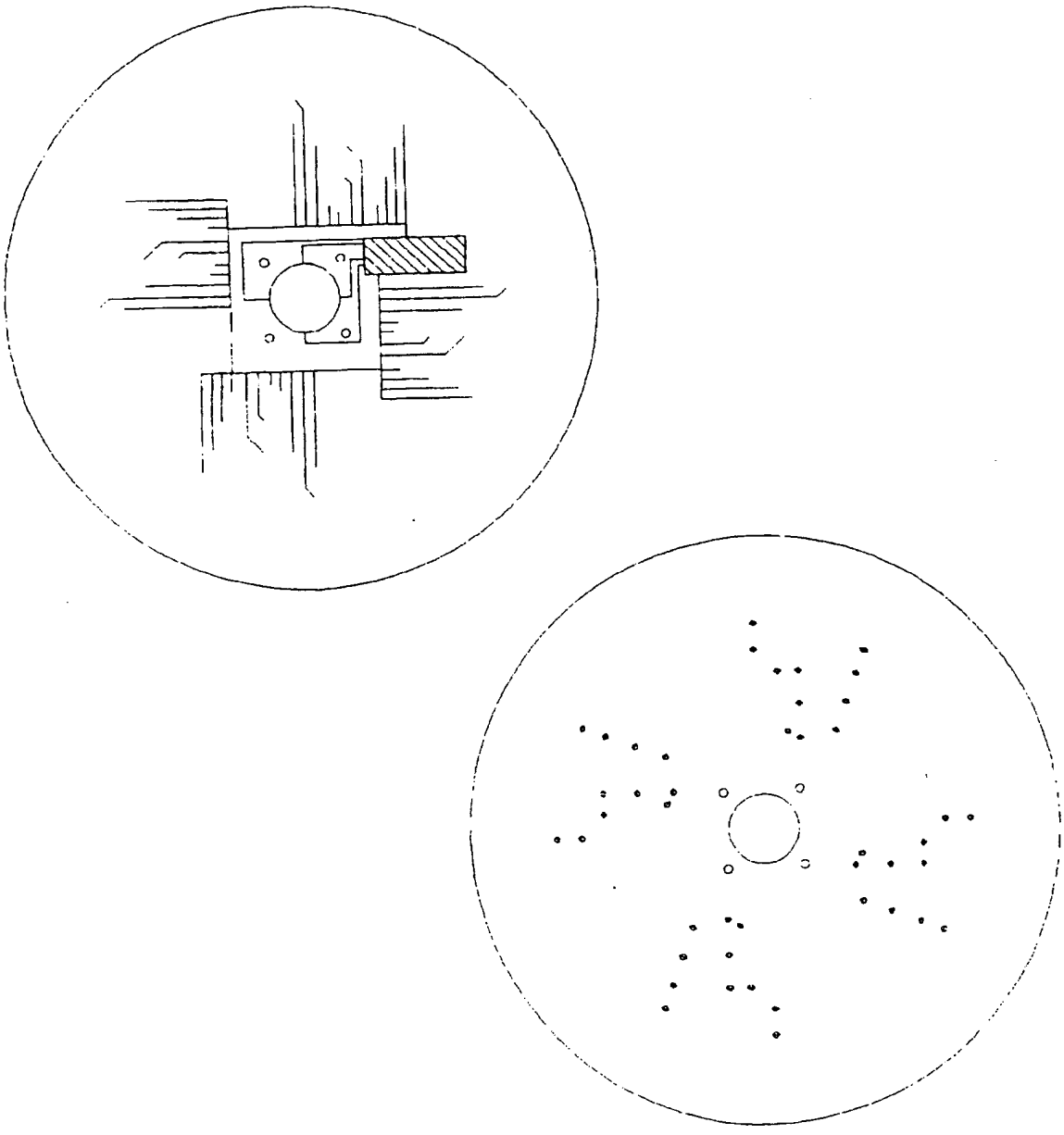


FIG. 17D

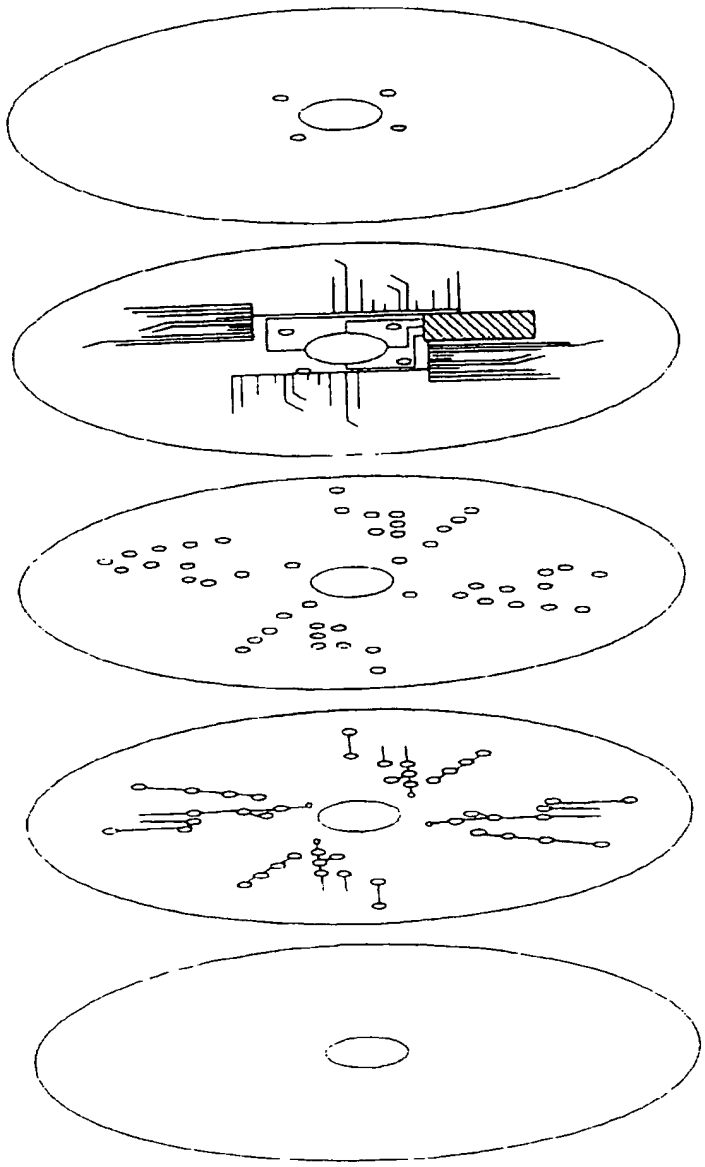


FIG. 17F

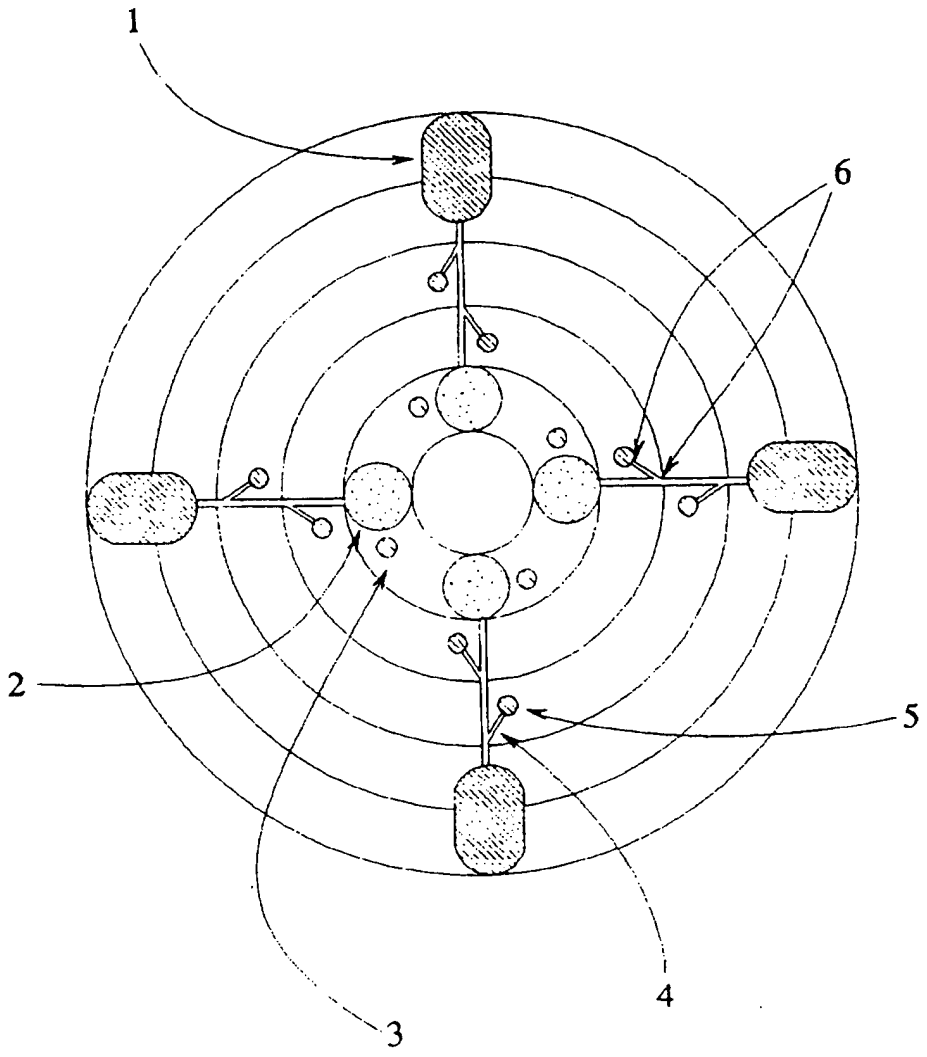


FIG. 17H

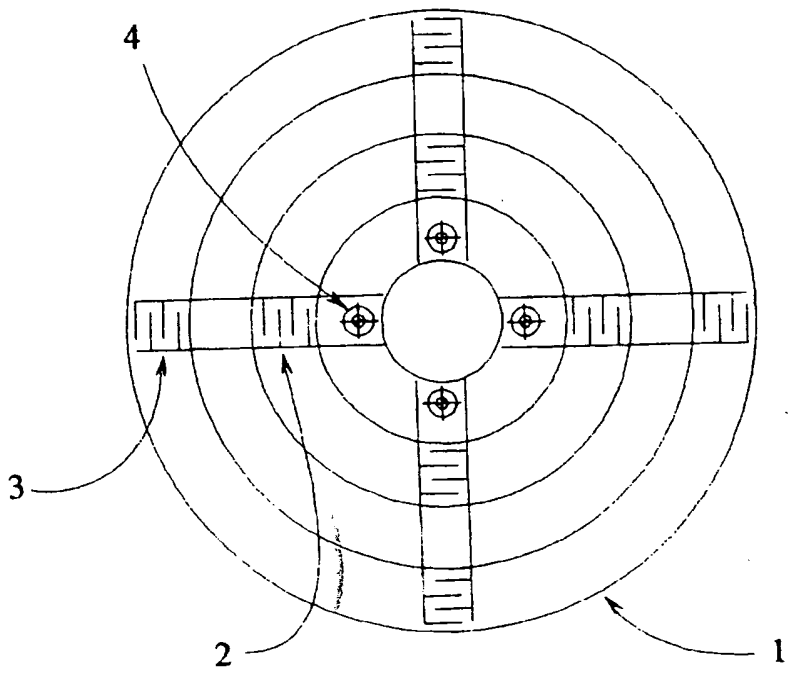


FIG. 17J

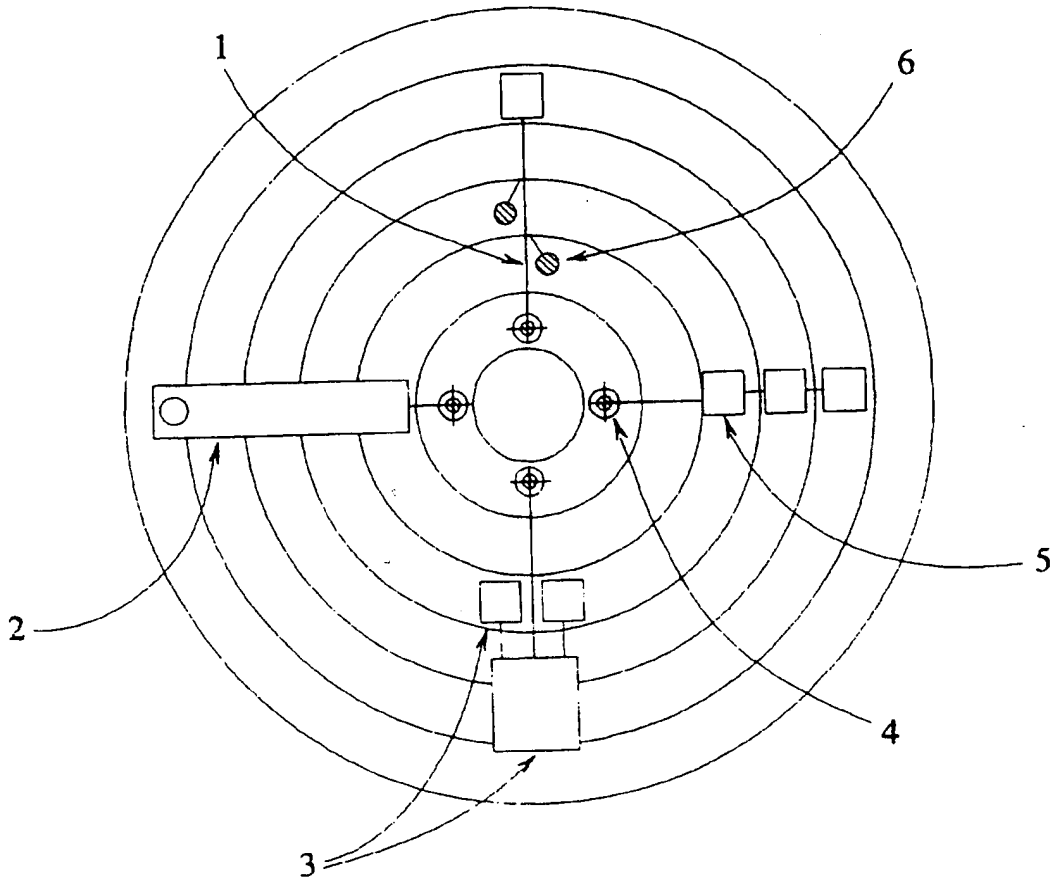


FIG. 17L

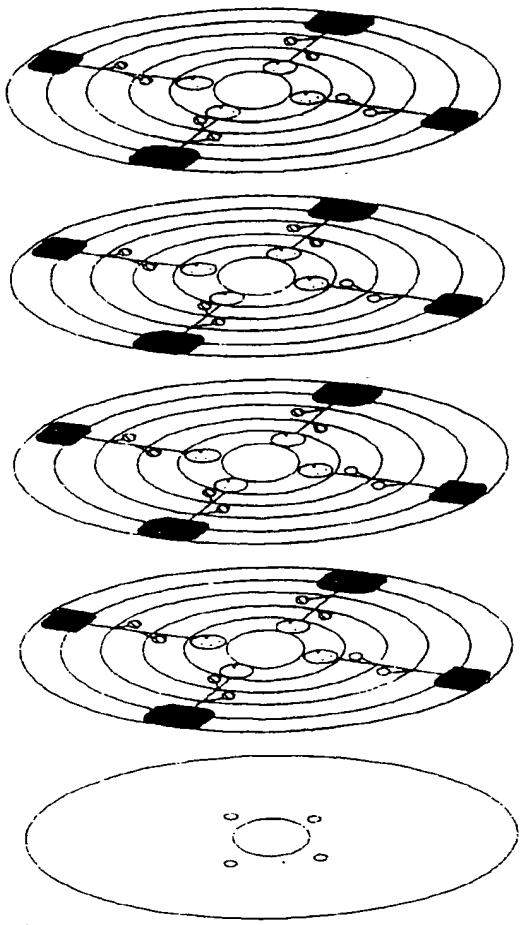


FIG. 17N

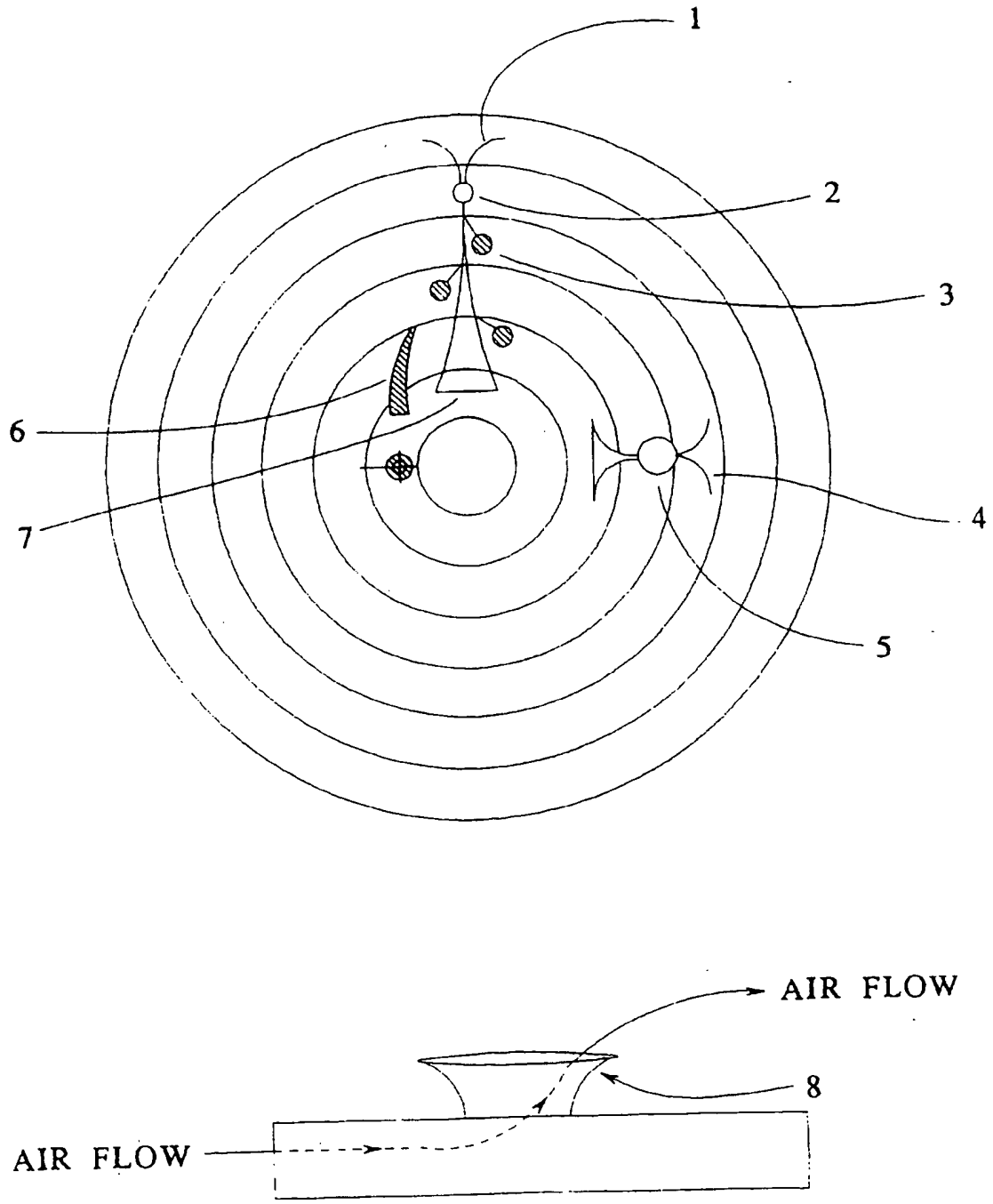


FIG. 17P

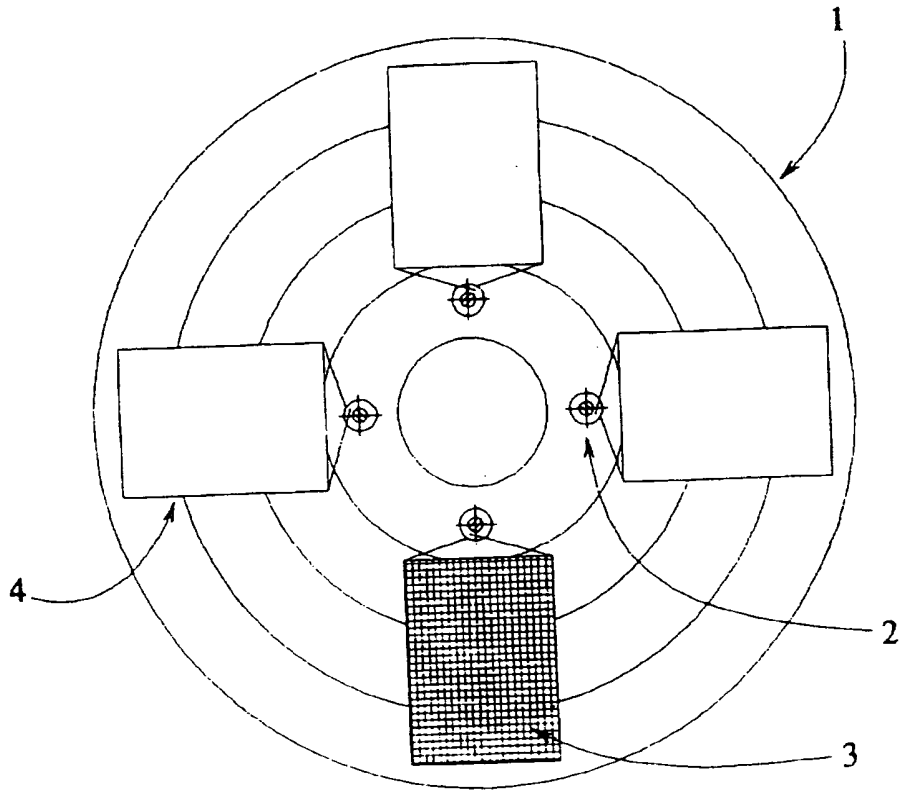
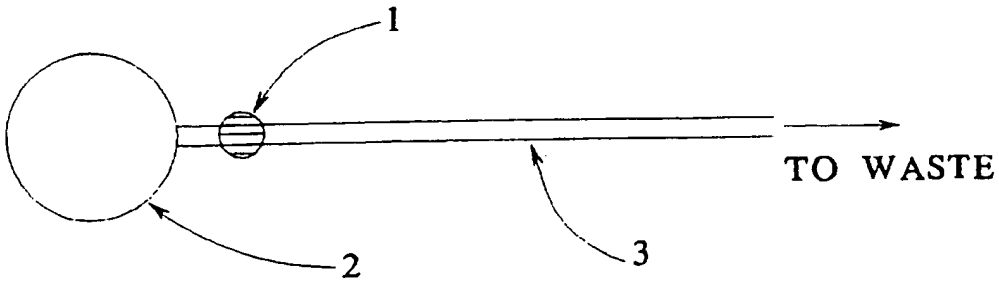


FIG. 17R



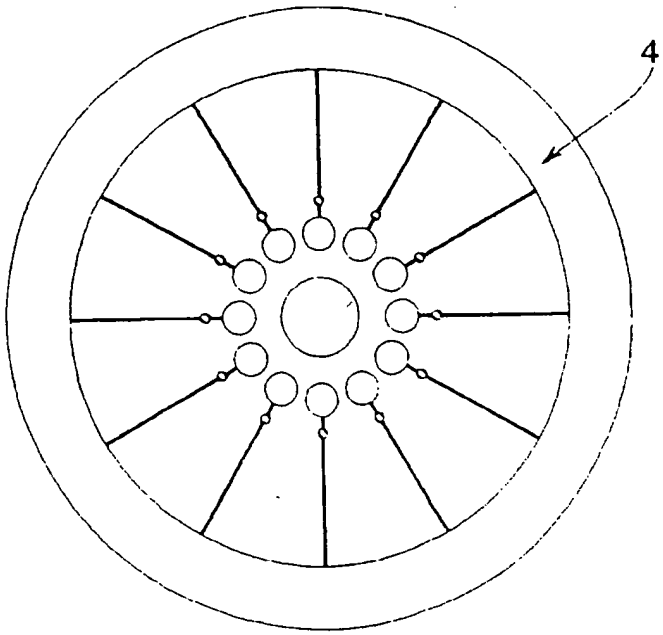


FIG. 19

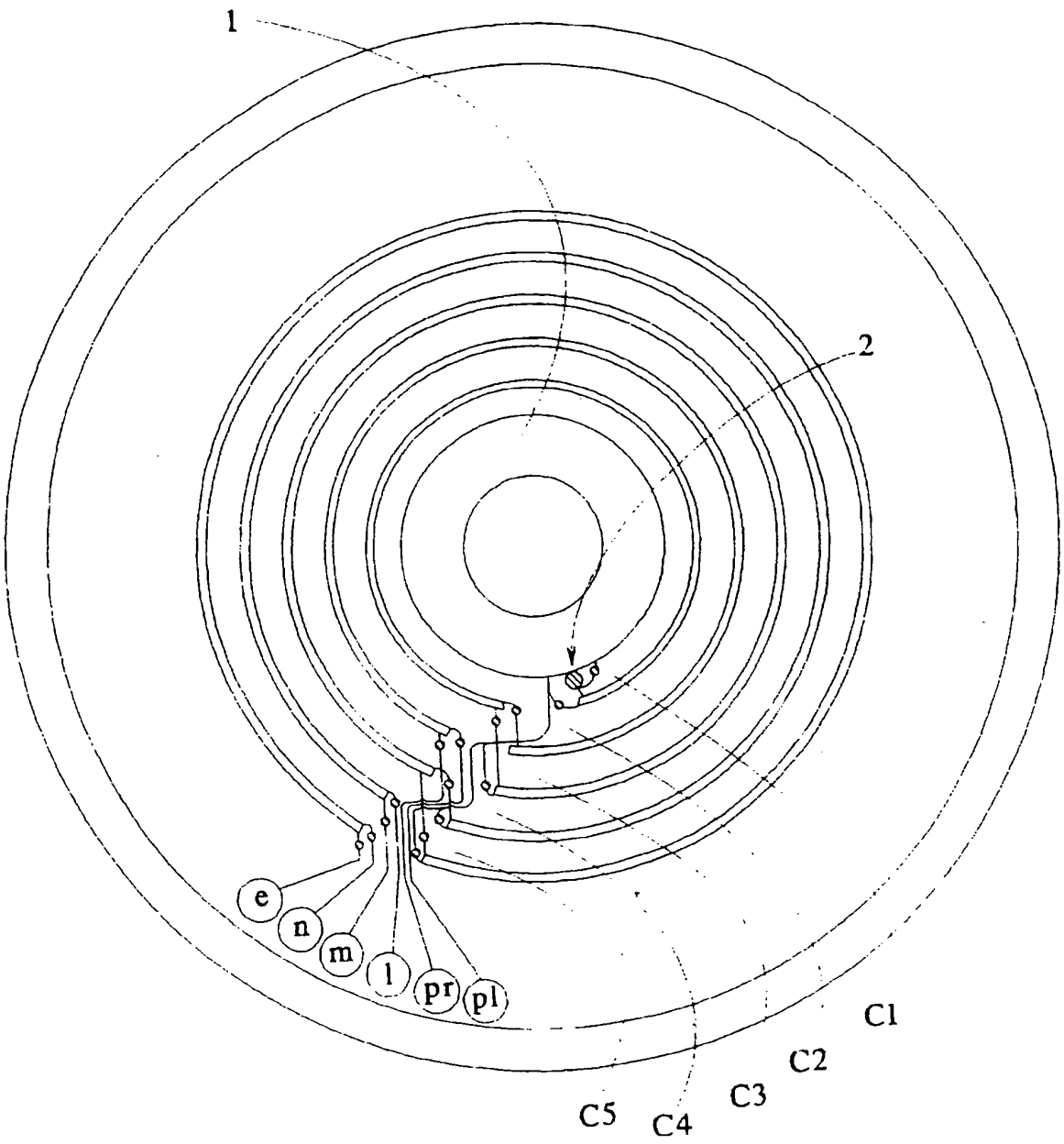


FIG. 21

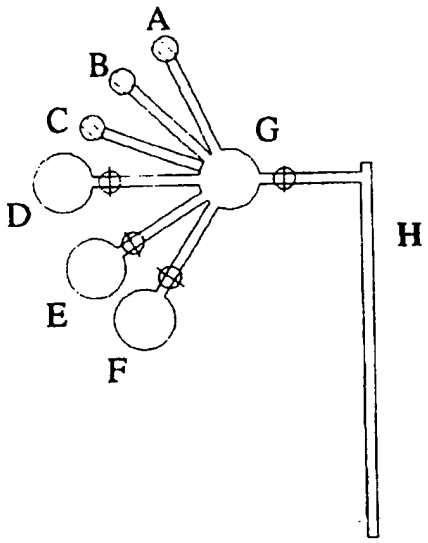


FIG. 22

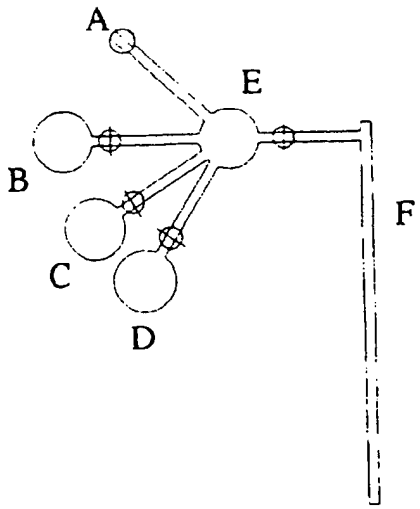


FIG. 23B

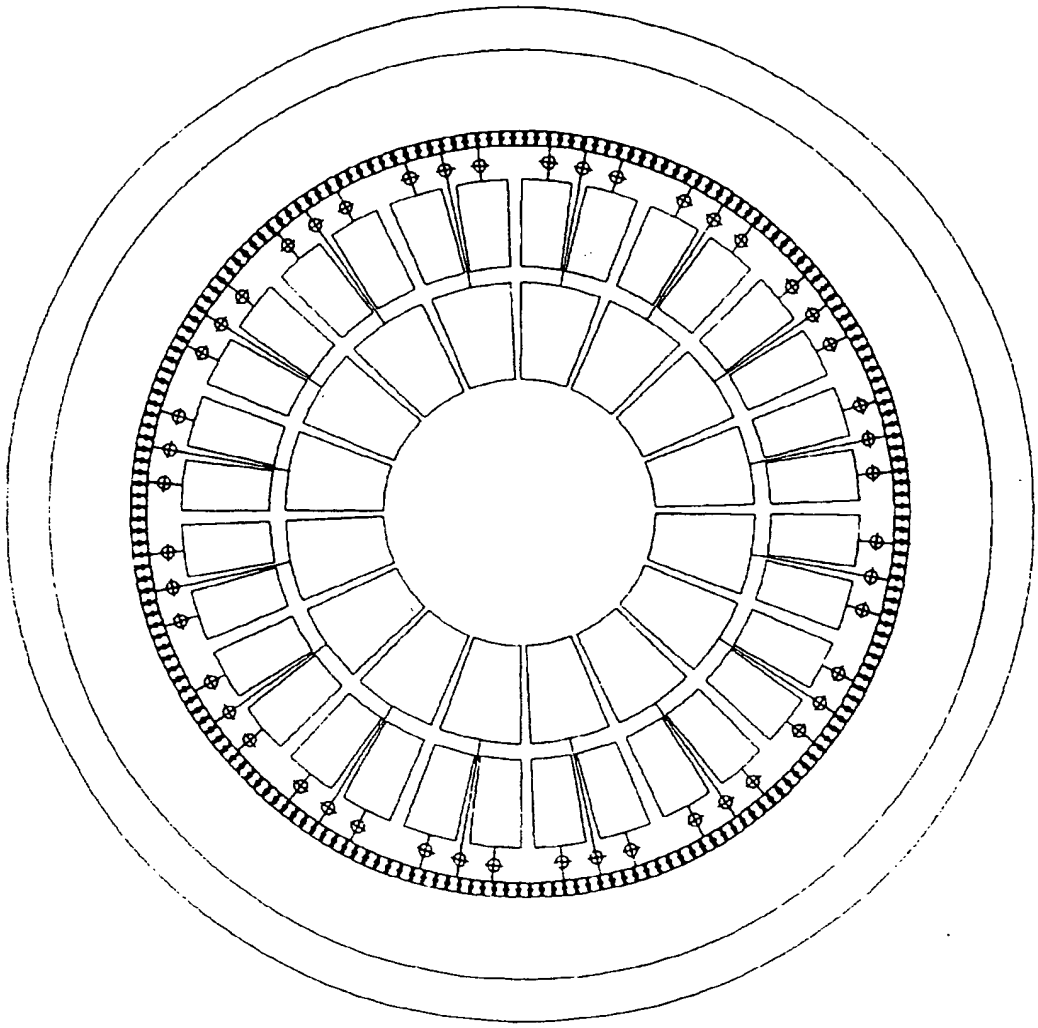


FIG. 25

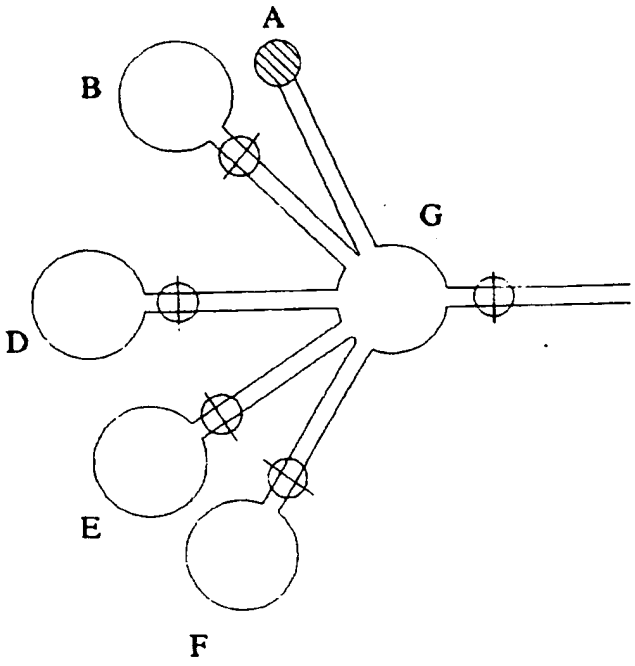


FIG. 27

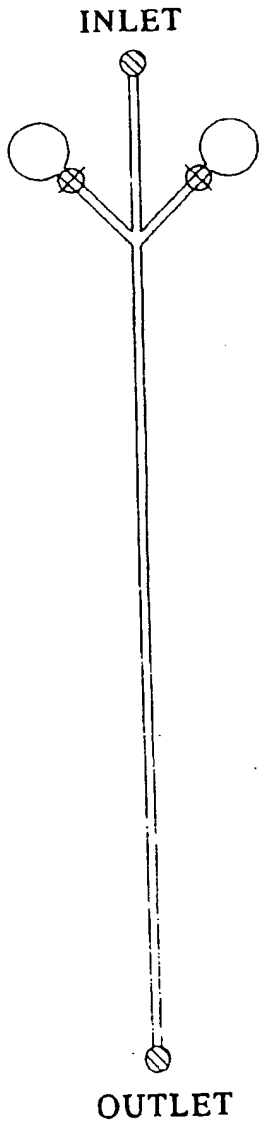


FIG. 29

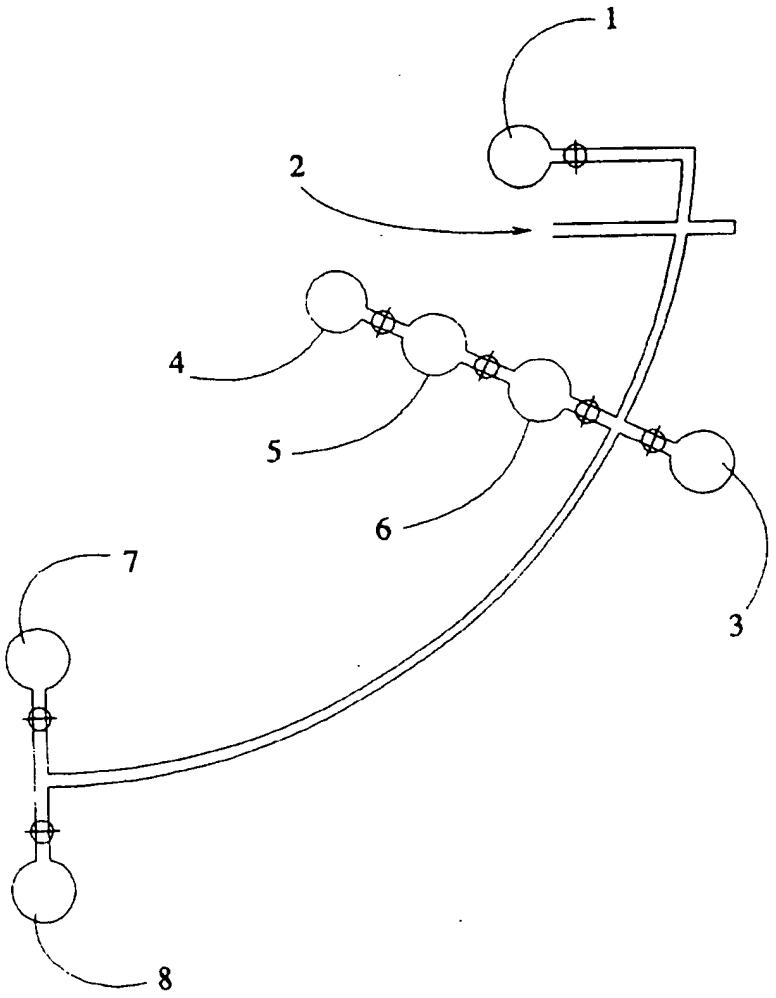


FIG. 31A

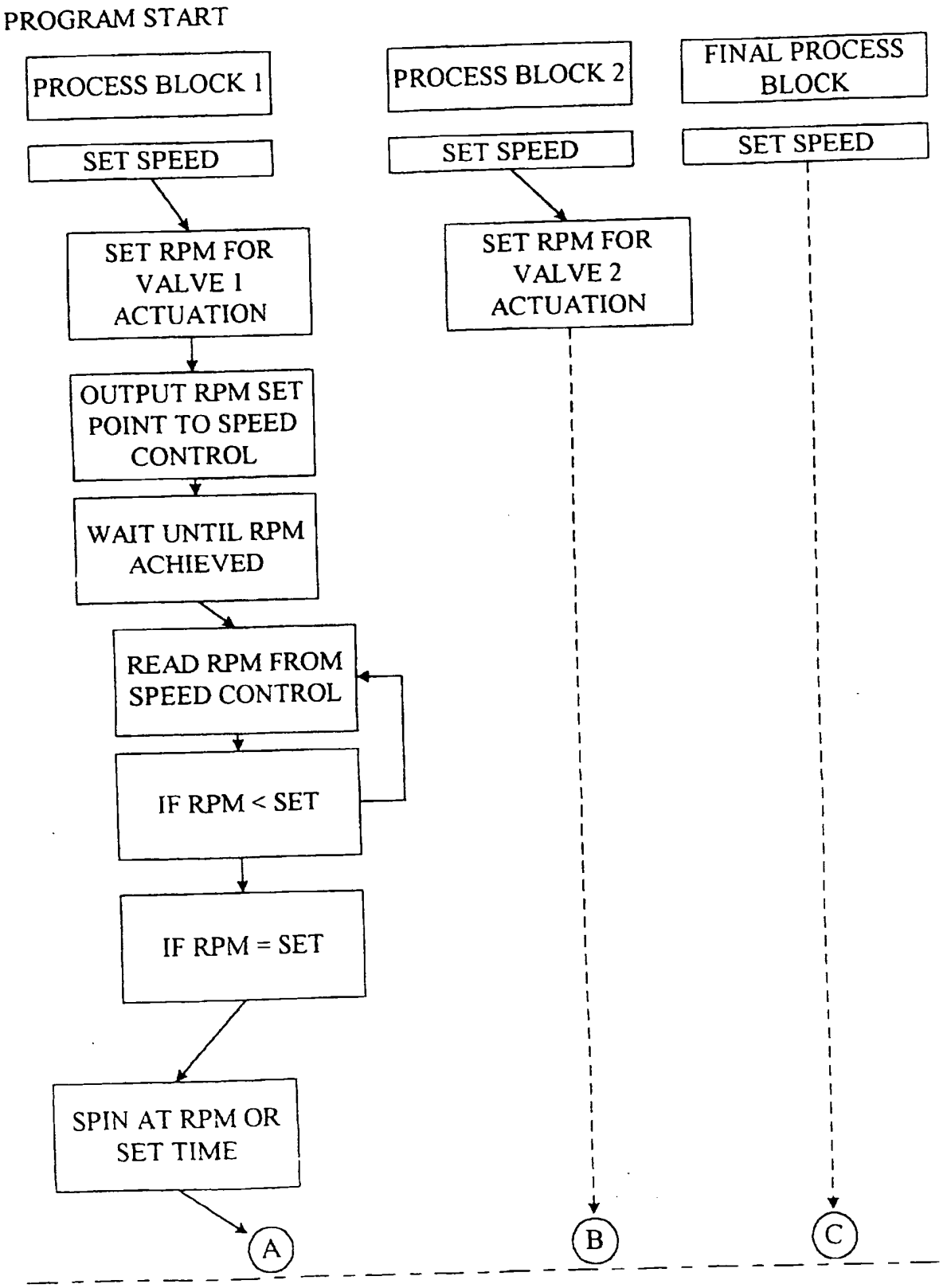
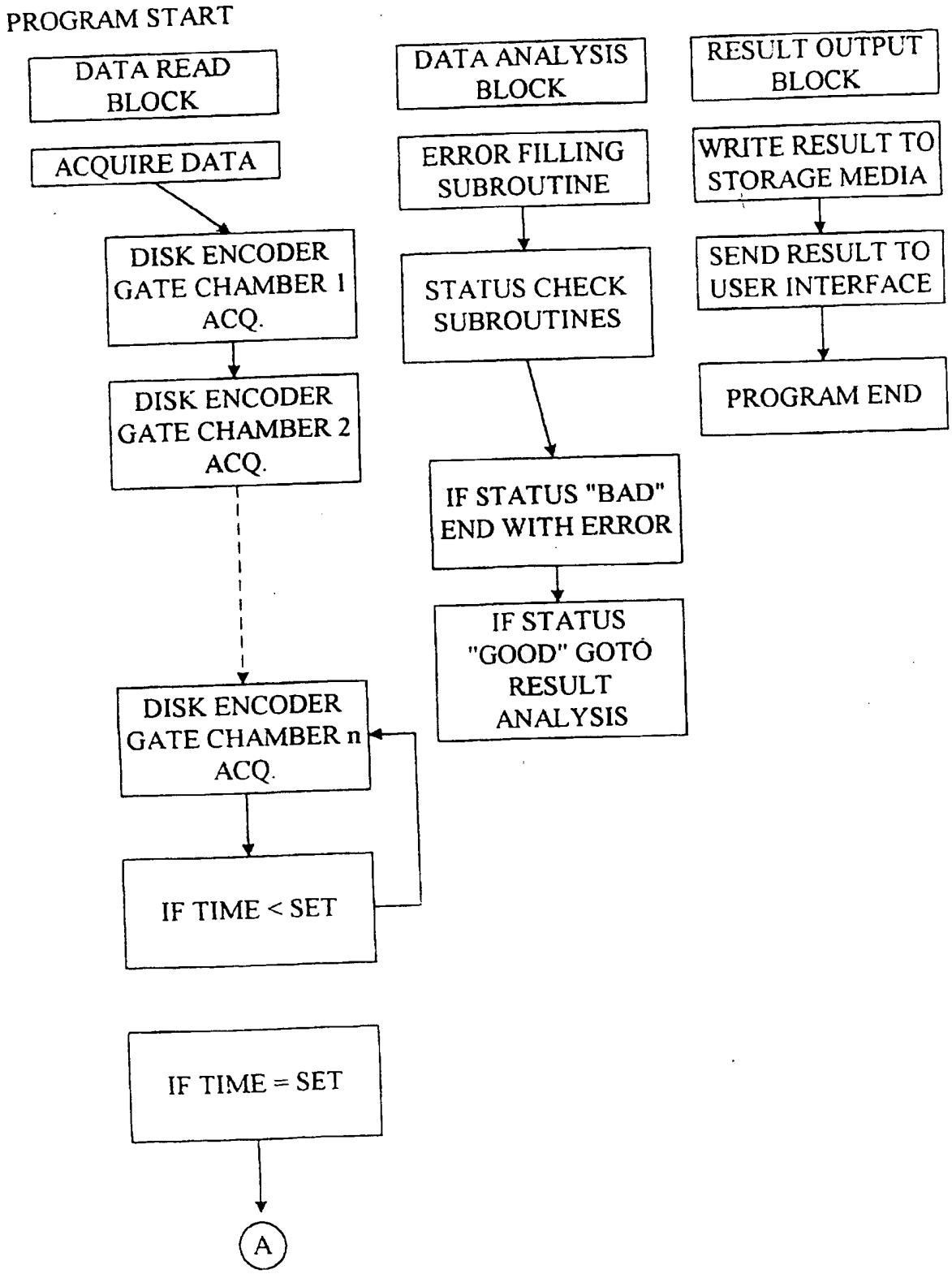

PROGRAM START
PROCESS BLOCK 1
SET SPEED
SET RPM FOR VALVE 1 ACTUATION
OUTPUT RPM SET POINT TO SPEED CONTROL
WAIT UNTIL RPM ACHIEVED
READ RPM FROM SPEED CONTROL
IF RPM < SET
IF RPM = SET
SPIN AT RPM OR SET TIME
A
PROCESS BLOCK 2
SET SPEED
SET RPM FOR VALVE 2 ACTUATION
B
FINAL PROCESS BLOCK
SET SPEED
C

FIG. 32A


PROGRAM START
DATA READ BLOCK
ACQUIRE DATA
DISK ENCODER GATE CHAMBER 1 ACQ.
DISK ENCODER GATE CHAMBER 2 ACQ.
DISK ENCODER GATE CHAMBER n ACQ.
IF TIME < SET
IF TIME = SET
A
DATA ANALYSIS BLOCK
ERROR FILLING SUBROUTINE
STATUS CHECK SUBROUTINES
IF STATUS "BAD" END WITH ERROR
IF STATUS "GOOD" GOTO RESULT ANALYSIS
RESULT OUTPUT BLOCK
WRITE RESULT TO STORAGE MEDIA
SEND RESULT TO USER INTERFACE
PROGRAM END

INTERNATIONAL SEARCH REPORT

Inter mal Application No
PCT/US 96/19514

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/07 B01L3/00 G01N33/487

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 417 305 A (IDEMITSU PETROCHEMICAL CO LTD) 20 March 1991	1-33, 35-50, 52-63, 78,82
A	see the whole document ---	79
Y	EP 0 616 218 A (HITACHI LTD) 21 September 1994	1-21, 30-33, 35-50, 52-63
	see column 7, line 54 - column 11, line 29 ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 March 1997	26.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bindon, C

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. onal Application No
PCT/US 96/19514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 417305 A	20-03-91	JP 3225278 A	04-10-91
		JP 2232563 A	14-09-90
		JP 6023767 B	30-03-94
		JP 2269969 A	05-11-90
		JP 6019359 B	16-03-94
		CA 2028829 A	08-09-90
		WO 9010875 A	20-09-90
EP 616218 A	21-09-94	JP 6265447 A	22-09-94
		US 5480614 A	02-01-96
WO 9322058 A	11-11-93	US 5304487 A	19-04-94
		US 5296375 A	22-03-94
		AT 140025 T	15-07-96
		AT 140880 T	15-08-96
		AU 4222393 A	29-11-93
		AU 4222593 A	29-11-93
		AU 4222693 A	29-11-93
		AU 4222793 A	29-11-93
		AU 4223593 A	29-11-93
		CA 2134474 A	11-11-93
		CA 2134475 A	11-11-93
		CA 2134476 A	11-11-93
		CA 2134478 A	11-11-93
		DE 69303483 D	08-08-96
		DE 69303483 T	06-02-97
		DE 69303898 D	05-09-96
		DE 69303898 T	20-02-97
		EP 0637996 A	15-02-95
		EP 0637997 A	15-02-95
		EP 0639223 A	22-02-95
		EP 0637998 A	15-02-95
		EP 0637999 A	15-02-95
		JP 7506430 T	13-07-95
		JP 7506431 T	13-07-95
		JP 7506256 T	13-07-95
		JP 7506257 T	13-07-95
		JP 7506258 T	13-07-95
		WO 9322053 A	11-11-93
		WO 9322054 A	11-11-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. .uonal Application No
PCT/US 96/19514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4940527 A		ES 2053622 T	01-08-94
		JP 1088147 A	03-04-89
		US 5001417 A	19-03-91
		US 5134359 A	28-07-92